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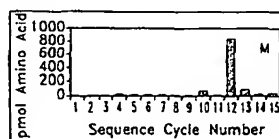
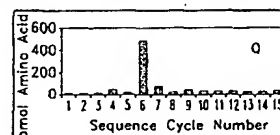
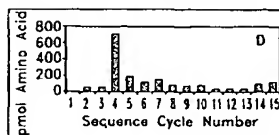
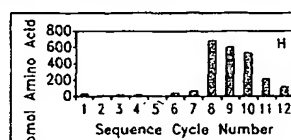
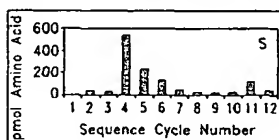
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- (54) Title: EPITOPE SEQUENCES

281 310
RGIAEAVGLPSIPVHPIGYYDAQKLLKMG



Pool sequencing of PSMA_281_310 Digested for 60 min by Proteasome

(57) Abstract: Disclosed herein are polypeptides, including epitopes, clusters, and antigens. Also disclosed are compositions including said polypeptides and methods for their use.

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EPITOPE SEQUENCES

Field of the Invention

5 The present invention generally relates to peptides, and nucleic acids encoding peptides, that are useful epitopes of target-associated antigens. More specifically, the invention relates to epitopes that have a high affinity for MHC class I and that are produced by target-specific proteasomes.

Description of the Related Art

Neoplasia and the Immune System

10 The neoplastic disease state commonly known as cancer is thought to result generally from a single cell growing out of control. The uncontrolled growth state typically results from a multi-step process in which a series of cellular systems fail, resulting in the genesis of a neoplastic cell. The resulting neoplastic cell rapidly reproduces itself, forms one or more tumors, and eventually may cause the death of the host.

15 Because the progenitor of the neoplastic cell shares the host's genetic material, neoplastic cells are largely unassailed by the host's immune system. During immune surveillance, the process in which the host's immune system surveys and localizes foreign materials, a neoplastic cell will appear to the host's immune surveillance machinery as a "self" cell.

Viruses and the Immune System

20 In contrast to cancer cells, virus infection involves the expression of clearly non-self antigens. As a result, many virus infections are successfully dealt with by the immune system with minimal clinical sequelae. Moreover, it has been possible to develop effective vaccines for many of those infections that do cause serious disease. A variety of vaccine approaches have been used successfully to combat various diseases. These approaches include subunit vaccines consisting of individual proteins produced through recombinant DNA technology. Notwithstanding these
25 advances, the selection and effective administration of minimal epitopes for use as viral vaccines has remained problematic.

30 In addition to the difficulties involved in epitope selection stands the problem of viruses that have evolved the capability of evading a host's immune system. Many viruses, especially viruses that establish persistent infections, such as members of the herpes and pox virus families, produce immunomodulatory molecules that permit the virus to evade the host's immune system. The effects of these immunomodulatory molecules on antigen presentation may be overcome by the targeting of select epitopes for administration as immunogenic compositions. To better understand the interaction of neoplastic cells and virally infected cells with the host's immune system, a discussion of the system's components follows below.

35 The immune system functions to discriminate molecules endogenous to an organism ("self" molecules) from material exogenous or foreign to the organism ("non-self" molecules). The

immune system has two types of adaptive responses to foreign bodies based on the components that mediate the response: a humoral response and a cell-mediated response. The humoral response is mediated by antibodies, while the cell-mediated response involves cells classified as lymphocytes. Recent anticancer and antiviral strategies have focused on mobilizing the host immune system as a means of anticancer or antiviral treatment or therapy.

The immune system functions in three phases to protect the host from foreign bodies: the cognitive phase, the activation phase, and the effector phase. In the cognitive phase, the immune system recognizes and signals the presence of a foreign antigen or invader in the body. The foreign antigen can be, for example, a cell surface marker from a neoplastic cell or a viral protein. Once the system is aware of an invading body, antigen specific cells of the immune system proliferate and differentiate in response to the invader-triggered signals. The last stage is the effector stage in which the effector cells of the immune system respond to and neutralize the detected invader.

An array of effector cells implements an immune response to an invader. One type of effector cell, the B cell, generates antibodies targeted against foreign antigens encountered by the host. In combination with the complement system, antibodies direct the destruction of cells or organisms bearing the targeted antigen. Another type of effector cell is the natural killer cell (NK cell), a type of lymphocyte having the capacity to spontaneously recognize and destroy a variety of virus infected cells as well as malignant cell types. The method used by NK cells to recognize target cells is poorly understood.

Another type of effector cell, the T cell, has members classified into three subcategories, each playing a different role in the immune response. Helper T cells secrete cytokines which stimulate the proliferation of other cells necessary for mounting an effective immune response, while suppressor T cells down-regulate the immune response. A third category of T cell, the cytotoxic T cell (CTL), is capable of directly lysing a targeted cell presenting a foreign antigen on its surface.

The Major Histocompatibility Complex and T Cell Target Recognition

T cells are antigen-specific immune cells that function in response to specific antigen signals. B lymphocytes and the antibodies they produce are also antigen-specific entities. However, unlike B lymphocytes, T cells do not respond to antigens in a free or soluble form. For a T cell to respond to an antigen, it requires the antigen to be processed to peptides which are then bound to a presenting structure encoded in the major histocompatibility complex (MHC). This requirement is called "MHC restriction" and it is the mechanism by which T cells differentiate "self" from "non-self" cells. If an antigen is not displayed by a recognizable MHC molecule, the T cell will not recognize and act on the antigen signal. T cells specific for a peptide bound to a recognizable MHC molecule bind to these MHC-peptide complexes and proceed to the next stages of the immune response.

There are two types of MHC, class I MHC and class II MHC. T Helper cells ($CD4^+$) predominately interact with class II MHC proteins while cytolytic T cells ($CD8^+$) predominately

interact with class I MHC proteins. Both classes of MHC protein are transmembrane proteins with a majority of their structure on the external surface of the cell. Additionally, both classes of MHC proteins have a peptide binding cleft on their external portions. It is in this cleft that small fragments of proteins, endogenous or foreign, are bound and presented to the extracellular environment.

5 Cells called "professional antigen presenting cells" (pAPCs) display antigens to T cells using the MHC proteins but additionally express various co-stimulatory molecules depending on the particular state of differentiation/activation of the pAPC. When T cells, specific for the peptide bound to a recognizable MHC protein, bind to these MHC-peptide complexes on pAPCs, the specific co-stimulatory molecules that act upon the T cell direct the path of differentiation/activation taken by the
10 T cell. That is, the co-stimulation molecules affect how the T cell will act on antigenic signals in future encounters as it proceeds to the next stages of the immune response.

As discussed above, neoplastic cells are largely ignored by the immune system. A great deal of effort is now being expended in an attempt to harness a host's immune system to aid in combating the presence of neoplastic cells in a host. One such area of research involves the formulation of
15 anticancer vaccines.

Anticancer Vaccines

Among the various weapons available to an oncologist in the battle against cancer is the immune system of the patient. Work has been done in various attempts to cause the immune system to combat cancer or neoplastic diseases. Unfortunately, the results to date have been
20 largely disappointing. One area of particular interest involves the generation and use of anticancer vaccines.

To generate a vaccine or other immunogenic composition, it is necessary to introduce to a subject an antigen or epitope against which an immune response may be mounted. Although neoplastic cells are derived from and therefore are substantially identical to normal cells on a genetic level, many neoplastic cells are known to present tumor-associated antigens (TuAAs). In
25 theory, these antigens could be used by a subject's immune system to recognize these antigens and attack the neoplastic cells. In reality, however, neoplastic cells generally appear to be ignored by the host's immune system.

A number of different strategies have been developed in an attempt to generate vaccines with activity against neoplastic cells. These strategies include the use of tumor-associated antigens as immunogens. For example, U.S. Patent No. 5,993,828, describes a method for producing an immune response against a particular subunit of the Urinary Tumor Associated Antigen by administering to a subject an effective dose of a composition comprising inactivated tumor cells having the Urinary Tumor Associated Antigen on the cell surface and at least one tumor associated
30 antigen selected from the group consisting of GM-2, GD-2, Fetal Antigen and Melanoma
35

Associated Antigen. Accordingly, this patent describes using whole, inactivated tumor cells as the immunogen in an anticancer vaccine.

Another strategy used with anticancer vaccines involves administering a composition containing isolated tumor antigens. In one approach, MAGE-A1 antigenic peptides were used as an immunogen. (See Chaux, P., *et al.*, "Identification of Five MAGE-A1 Epitopes Recognized by Cytolytic T Lymphocytes Obtained by *In Vitro* Stimulation with Dendritic Cells Transduced with MAGE-A1," J. Immunol., 163(5):2928-2936 (1999)). There have been several therapeutic trials using MAGE-A1 peptides for vaccination, although the effectiveness of the vaccination regimes was limited. The results of some of these trials are discussed in Vose, J.M., "Tumor Antigens Recognized by T Lymphocytes," 10th European Cancer Conference, Day 2, Sept. 14, 1999.

In another example of tumor associated antigens used as vaccines, Scheinberg, *et al.* treated 12 chronic myelogenous leukemia (CML) patients already receiving interferon (IFN) or hydroxyurea with 5 injections of class I-associated bcr-abl peptides with a helper peptide plus the adjuvant QS-21. Scheinberg, D.A., *et al.*, "BCR-ABL Breakpoint Derived Oncogene Fusion Peptide Vaccines Generate Specific Immune Responses in Patients with Chronic Myelogenous Leukemia (CML) [Abstract 1665], American Society of Clinical Oncology 35th Annual Meeting, Atlanta (1999). Proliferative and delayed type hypersensitivity (DTH) T cell responses indicative of T-helper activity were elicited, but no cytolytic killer T cell activity was observed within the fresh blood samples.

Additional examples of attempts to identify TuAAs for use as vaccines are seen in the recent work of Cebon, *et al.* and Scheibenbogen, *et al.* Cebon, *et al.* immunized patients with metastatic melanoma using intradermally administered MART-1₂₆₋₃₅ peptide with IL-12 in increasing doses given either subcutaneously or intravenously. Of the first 15 patients, 1 complete remission, 1 partial remission, and 1 mixed response were noted. Immune assays for T cell generation included DTH, which was seen in patients with or without IL-12. Positive CTL assays were seen in patients with evidence of clinical benefit, but not in patients without tumor regression. Cebon, *et al.*, "Phase I Studies of Immunization with Melan-A and IL-12 in HLA A2+ Positive Patients with Stage III and IV Malignant Melanoma," [Abstract 1671], American Society of Clinical Oncology 35th Annual Meeting, Atlanta (1999).

Scheibenbogen, *et al.* immunized 18 patients with 4 HLA class I restricted tyrosinase peptides, 16 with metastatic melanoma and 2 adjuvant patients. Scheibenbogen, *et al.*, "Vaccination with Tyrosinase peptides and GM-CSF in Metastatic Melanoma: a Phase II Trial," [Abstract 1680], American Society of Clinical Oncology 35th Annual Meeting, Atlanta (1999). Increased CTL activity was observed in 4/15 patients, 2 adjuvant patients, and 2 patients with evidence of tumor regression. As in the trial by Cebon, *et al.*, patients with progressive disease did

not show boosted immunity. In spite of the various efforts expended to date to generate efficacious anticancer vaccines, no such composition has yet been developed.

Antiviral Vaccines

5 Vaccine strategies to protect against viral diseases have had many successes. Perhaps the most notable of these is the progress that has been made against the disease small pox, which has been driven to extinction. The success of the polio vaccine is of a similar magnitude.

10 Viral vaccines can be grouped into three classifications: live attenuated virus vaccines, such as vaccinia for small pox, the Sabin poliovirus vaccine, and measles mumps and rubella; whole killed or inactivated virus vaccines, such as the Salk poliovirus vaccine, hepatitis A. virus vaccine and the typical influenza virus vaccines; and subunit vaccines, such as hepatitis B. Due to their lack of a complete viral genome, subunit vaccines offer a greater degree of safety than those based on whole viruses.

15 The paradigm of a successful subunit vaccine is the recombinant hepatitis B vaccine based on the viruses envelope protein. Despite much academic interest in pushing the reductionist subunit concept beyond single proteins to individual epitopes, the efforts have yet to bear much fruit. Viral vaccine research has also concentrated on the induction of an antibody response although cellular responses also occur. However, many of the subunit formulations are particularly poor at generating a CTL response.

Summary of the Invention

20 Previous methods of priming professional antigen presenting cells (pAPCs) to display target cell epitopes have relied simply on causing the pAPCs to express target-associated antigens (TAAs), or epitopes of those antigens which are thought to have a high affinity for MHC I molecules. However, the proteasomal processing of such antigens results in presentation of epitopes on the pAPC that do not correspond to the epitopes present on the target cells.

25 Using the knowledge that an effective cellular immune response requires that pAPCs present the same epitope that is presented by the target cells, the present invention provides epitopes that have a high affinity for MHC I, and that correspond to the processing specificity of the housekeeping proteasome, which is active in peripheral cells. These epitopes thus correspond to those presented on target cells. The use of such epitopes in vaccines can activate the cellular
30 immune response to recognize the correctly processed TAA and can result in removal of target cells that present such epitopes. In some embodiments, the housekeeping epitopes provided herein can be used in combination with immune epitopes, generating a cellular immune response that is competent to attack target cells both before and after interferon induction. In other embodiments the epitopes are useful in the diagnosis and monitoring of the target-associated disease and in the
35 generation of immunological reagents for such purposes.

Embodiments of the invention relate to isolated epitopes and antigens or polypeptides that comprise the epitopes. Preferred embodiments include an epitope or antigen having the sequence as disclosed in TABLE 1. Other embodiments can include an epitope cluster comprising a polypeptide from Table 1. Further, embodiments include a polypeptide having substantial similarity to the already mentioned epitopes, antigens, or clusters. Other preferred embodiments include a polypeptide having functional similarity to any of the above. Still further embodiments relate to a nucleic acid encoding the polypeptide of any of the epitopes, clusters, antigens, and polypeptides from Table 1 and mentioned herein.

The epitope can be immunologically active. The polypeptide comprising the epitope can be less than about 30 amino acids in length, more preferably, the polypeptide is 8 to 10 amino acids in length, for example. Substantial or functional similarity can include addition of at least one amino acid, for example, and the at least one additional amino acid can be at an N-terminus of the polypeptide. The substantial or functional similarity can include a substitution of at least one amino acid.

The epitope, cluster, or polypeptide comprising the same can have affinity to an HLA-A2 molecule. The affinity can be determined by an assay of binding, by an assay of restriction of epitope recognition, by a prediction algorithm, and the like. The epitope, cluster, or polypeptide comprising the same can have affinity to an HLA-B7, HLA-B51 molecule, and the like.

In preferred embodiments the polypeptide can be a housekeeping epitope. The epitope or polypeptide can correspond to an epitope displayed on a tumor cell, to an epitope displayed on a neovasculature cell, and the like. The epitope or polypeptide can be an immune epitope. The epitope, cluster and/or polypeptide can be a nucleic acid.

Other embodiments relate to pharmaceutical compositions comprising the polypeptides, including an epitope from Table 1, a cluster, or a polypeptide comprising the same and a pharmaceutically acceptable adjuvant, carrier, diluent, excipient, and the like. The adjuvant can be a polynucleotide. The polynucleotide can include a dinucleotide. The dinucleotide can be CpG, for example. The adjuvant can be encoded by a polynucleotide. The adjuvant can be a cytokine and the cytokine can be, for example, GM-CSF.

The pharmaceutical compositions can further include a professional antigen-presenting cell (pAPC). The pAPC can be a dendritic cell, for example. The pharmaceutical composition can further include a second epitope. The second epitope can be a polypeptide. The second epitope can be a nucleic acid. The second epitope can be a housekeeping epitope, an immune epitope, and the like.

Still further embodiments relate to pharmaceutical compositions that include any of the nucleic acids discussed herein, including those that encode polypeptides that comprise epitopes or

antigens from Table 1. Such compositions can include a pharmaceutically acceptable adjuvant, carrier, diluent, excipient, and the like.

5 Other embodiments relate to recombinant constructs that include such a nucleic acid as described herein, including those that encode polypeptides that comprise epitopes or antigens from Table 1. The constructs can further include a plasmid, a viral vector, an artificial chromosome, and the like. The construct can further include a sequence encoding at least one feature, such as for example, a second epitope, an IRES, an ISS, an NIS, ubiquitin.

10 Further embodiments relate to purified antibodies that specifically bind to at least one of the epitopes in Table 1A. Other embodiments relate to purified antibodies that specifically bind to a peptide-MHC protein complex comprising an epitope disclosed in Table 1A or any other suitable epitope. The antibody from any embodiment can be a monoclonal antibody.

Still other embodiments relate to multimeric MHC-peptide complexes that include an epitope, such as, for example, an epitope disclosed in Table 1.

15 Embodiments relate to isolated T cells expressing a T cell receptor specific for an MHC-peptide complex. The complex can include an epitope, such as, for example, an epitope disclosed in Table 1 of claim 1. The T cell can be produced by an *in vitro* immunization. The T cell can be isolated from an immunized animal. Embodiments relate to T cell clones, including cloned T cells, such as those discussed above. Embodiments also relate to polyclonal population of T cells. Such populations can include a T cell, as described above, for example.

20 Still further embodiments relate to pharmaceutical compositions that include a T cell, such as those described above, for example, and a pharmaceutically acceptable adjuvant, carrier, diluent, excipient, and the like.

25 Embodiments of the invention relate to isolated protein molecules comprising the binding domain of a T cell receptor specific for an MHC-peptide complex. The complex can include an epitope disclosed in Table 1. The protein can be multivalent. Other embodiments relate to isolated nucleic acids encoding such proteins. Still further embodiments relate to recombinant constructs that include such nucleic acids.

30 Other embodiments of the invention relate to host cells expressing the recombinant construct described herein, including constructs encoding an epitope, cluster or polypeptide comprising the same, disclosed in Table 1, for example. The host cell can be a dendritic cell, macrophage, tumor cell, tumor-derived cell, and the like. The host cell can be a bacterium, fungus, protozoan and the like. Embodiments also relate to pharmaceutical compositions that include a host cell, such as those discussed herein, and a pharmaceutically acceptable adjuvant, carrier, diluent, excipient, and the like.

35 Still other embodiments relate to vaccines or immunotherapeutic compositions that include at least one component, such as, for example, an epitope disclosed in Table 1 or otherwise

described herein; a cluster that includes such an epitope, an antigen or polypeptide that includes such an epitope; a composition described above and herein; a construct, a T cell, or a host cell as described above and herein.

Further embodiments relate to methods of treating an animal. The methods can include administering to an animal a vaccine or immunotherapeutic composition, including those disclosed above and herein. The administering step can include a mode of delivery, such as, for example, transdermal, intranodal, perinodal, oral, intravenous, intradermal, intramuscular, intraperitoneal, mucosal, aerosol inhalation, instillation, and the like. The method can further include a step of assaying to determine a characteristic indicative of a state of a target cell or target cells. The method can include a first assaying step and a second assaying step, wherein the first assaying step precedes the administering step, and wherein the second assaying step follows the administering step. The method can further include a step of comparing the characteristic determined in the first assaying step with the characteristic determined in the second assaying step to obtain a result. The result can be for example, evidence of an immune response, a diminution in number of target cells, a loss of mass or size of a tumor comprising target cells, a decrease in number or concentration of an intracellular parasite infecting target cells, and the like.

Embodiments relate to methods of evaluating immunogenicity of a vaccine or immunotherapeutic composition. The methods can include administering to an animal a vaccine or immunotherapeutic, such as those described above and elsewhere herein, and evaluating immunogenicity based on a characteristic of the animal. The animal can be HLA-transgenic.

Other embodiments relate to methods of evaluating immunogenicity that include *in vitro* stimulation of a T cell with the vaccine or immunotherapeutic composition, such as those described above and elsewhere herein, and evaluating immunogenicity based on a characteristic of the T cell. The stimulation can be a primary stimulation.

Still further embodiments relate to methods of making a passive/adoptive immunotherapeutic. The methods can include combining a T cell or a host cell, such as those described above and elsewhere herein, with a pharmaceutically acceptable adjuvant, carrier, diluent, excipient, and the like.

Other embodiments relate to methods of determining specific T cell frequency, and can include the step of contacting T cells with a MHC-peptide complex comprising an epitope disclosed in Table 1, or a complex comprising a cluster or antigen comprising such an epitope. The contacting step can include at least one feature, such as, for example, immunization, restimulation, detection, enumeration, and the like. The method can further include ELISPOT analysis, limiting dilution analysis, flow cytometry, *in situ* hybridization, the polymerase chain reaction, any combination thereof, and the like.

Embodiments relate to methods of evaluating immunologic response. The methods can include the above-described methods determining specific T cell frequency carried out prior to and subsequent to an immunization step.

5 Another embodiment relates to methods of evaluating immunologic response. The methods can include determining frequency, cytokine production, or cytolytic activity of T cells, prior to and subsequent to a step of stimulation with MHC-peptide complexes comprising an epitope, such as, for example an epitope from Table 1, a cluster or a polypeptide comprising such an epitope.

10 Further embodiments relate to methods of diagnosing a disease. The methods can include contacting a subject tissue with at least one component, including, for example, a T cell, a host cell, an antibody, a protein, including those described above and elsewhere herein; and diagnosing the disease based on a characteristic of the tissue or of the component. The contacting step can take place *in vivo*. The contacting step can take place *in vitro*.

15 Still other embodiments relate to methods of making a vaccine. The methods can include combining at least one component, an epitope, a composition, a construct, a T cell, a host cell; including any of those described above and elsewhere herein, with a pharmaceutically acceptable adjuvant, carrier, diluent, excipient, and the like.

20 Embodiments relate to computer readable media having recorded thereon the sequence of any one of SEQ ID NOS: 1 -602, in a machine having a hardware or software that calculates the physical, biochemical, immunologic, or molecular genetic properties of a molecule embodying said sequence.

25 Still other embodiments relate to methods of treating an animal. The methods can include combining the method of treating an animal that includes administering to the animal a vaccine or immunotherapeutic composition, such as described above and elsewhere herein, combined with at least one mode of treatment, including, for example, radiation therapy, chemotherapy, biochemotherapy, surgery, and the like.

30 Further embodiments relate to isolated polypeptides that include an epitope cluster from a target-associated antigen having the sequence as disclosed in any one of Tables 25-44, wherein the amino acid sequence includes not more than about 80% of the amino acid sequence of the antigen.

Other embodiments relate to vaccines or immunotherapeutic products that include an isolated peptide as described above and elsewhere herein. Still other embodiments relate to isolated polynucleotides encoding a polypeptide as described above and elsewhere herein. Other embodiments relate vaccines or immunotherapeutic products that include these polynucleotides. The polynucleotide can be DNA or RNA.

35

Brief Description of the Drawings

Figure 1 is a sequence alignment of NY-ESO-1 and several similar protein sequences.

Figure 2 graphically represents a plasmid vaccine backbone useful for delivering nucleic acid-encoded epitopes.

5 Figures 3A and 3B are FACS profiles showing results of HLA-A2 binding assays for tyrosinase₂₀₇₋₂₁₅ and tyrosinase₂₀₈₋₂₁₆.

Figure 4 is a T=120 min. time point mass spectrum of the fragments produced by proteasomal cleavage of SSX-2₃₁₋₆₈.

Figure 5 shows a binding curve for HLA-A2:SSX-2₄₁₋₄₉ with controls.

10 Figure 6 shows specific lysis of SSX-2₄₁₋₄₉-pulsed targets by CTL from SSX-2₄₁₋₄₉-immunized HLA-A2 transgenic mice.

Figure 7A, B, and C show results of N-terminal pool sequencing of a T=60 min. time point aliquot of the PSMA₁₆₃₋₁₉₂ proteasomal digest.

15 Figure 8 shows binding curves for HLA-A2:PSMA₁₆₈₋₁₇₇ and HLA-A2:PSMA₂₈₈₋₂₉₇ with controls.

Figure 9 shows results of N-terminal pool sequencing of a T=60 min. time point aliquot of the PSMA₂₈₁₋₃₁₀ proteasomal digest.

Figure 10 shows binding curves for HLA-A2:PSMA₄₆₁₋₄₆₉, HLA-A2:PSMA₄₆₀₋₄₆₉, and HLA-A2:PSMA₆₆₃₋₆₇₁, with controls.

20 Figure 11 shows the results of a γ -IFN-based ELISPOT assay detecting PSMA₄₆₃₋₄₇₁-reactive HLA-A1⁺ CD8⁺ T cells.

Figure 12 shows blocking of reactivity of the T cells used in figure 10 by anti-HLA-A1 mAb, demonstrating HLA-A1-restricted recognition.

Figure 13 shows a binding curve for HLA-A2:PSMA₆₆₃₋₆₇₁, with controls.

25 Figure 14 shows a binding curve for HLA-A2:PSMA₆₆₂₋₆₇₁, with controls.

Figure 15. Comparison of anti-peptide CTL responses following immunization with various doses of DNA by different routes of injection.

Figure 16. Growth of transplanted gp33 expressing tumor in mice immunized by i.ln. injection of gp33 epitope-expressing, or control, plasmid.

30 Figure 17. Amount of plasmid DNA detected by real-time PCR in injected or draining lymph nodes at various times after i.ln. of i.m. injection, respectively.

Detailed Description of the Preferred Embodiment

Definitions

35 Unless otherwise clear from the context of the use of a term herein, the following listed terms shall generally have the indicated meanings for purposes of this description.

PROFESSIONAL ANTIGEN-PRESENTING CELL (pAPC) – a cell that possesses T cell costimulatory molecules and is able to induce a T cell response. Well characterized pAPCs include dendritic cells, B cells, and macrophages.

PERIPHERAL CELL – a cell that is not a pAPC.

5 HOUSEKEEPING PROTEASOME – a proteasome normally active in peripheral cells, and generally not present or not strongly active in pAPCs.

IMMUNE PROTEASOME – a proteasome normally active in pAPCs; the immune proteasome is also active in some peripheral cells in infected tissues.

10 EPITOPE – a molecule or substance capable of stimulating an immune response. In preferred embodiments, epitopes according to this definition include but are not necessarily limited to a polypeptide and a nucleic acid encoding a polypeptide, wherein the polypeptide is capable of stimulating an immune response. In other preferred embodiments, epitopes according to this definition include but are not necessarily limited to peptides presented on the surface of cells, the peptides being non-covalently bound to the binding cleft of class I MHC, such that they can
15 interact with T cell receptors.

MHC EPITOPE – a polypeptide having a known or predicted binding affinity for a mammalian class I or class II major histocompatibility complex (MHC) molecule.

HOUSEKEEPING EPITOPE – In a preferred embodiment, a housekeeping epitope is defined as a polypeptide fragment that is an MHC epitope, and that is displayed on a cell in which
20 housekeeping proteasomes are predominantly active. In another preferred embodiment, a housekeeping epitope is defined as a polypeptide containing a housekeeping epitope according to the foregoing definition, that is flanked by one to several additional amino acids. In another preferred embodiment, a housekeeping epitope is defined as a nucleic acid that encodes a housekeeping epitope according to the foregoing definitions.

25 IMMUNE EPITOPE – In a preferred embodiment, an immune epitope is defined as a polypeptide fragment that is an MHC epitope, and that is displayed on a cell in which immune proteasomes are predominantly active. In another preferred embodiment, an immune epitope is defined as a polypeptide containing an immune epitope according to the foregoing definition, that is flanked by one to several additional amino acids. In another preferred embodiment, an immune
30 epitope is defined as a polypeptide including an epitope cluster sequence, having at least two polypeptide sequences having a known or predicted affinity for a class I MHC. In yet another preferred embodiment, an immune epitope is defined as a nucleic acid that encodes an immune epitope according to any of the foregoing definitions.

35 TARGET CELL – a cell to be targeted by the vaccines and methods of the invention. Examples of target cells according to this definition include but are not necessarily limited to: a

neoplastic cell and a cell harboring an intracellular parasite, such as, for example, a virus, a bacterium, or a protozoan.

TARGET-ASSOCIATED ANTIGEN (TAA) – a protein or polypeptide present in a target cell.

5 TUMOR-ASSOCIATED ANTIGENS (TuAA) – a TAA, wherein the target cell is a neoplastic cell.

HLA EPITOPE – a polypeptide having a known or predicted binding affinity for a human class I or class II HLA complex molecule.

10 ANTIBODY – a natural immunoglobulin (Ig), poly- or monoclonal, or any molecule composed in whole or in part of an Ig binding domain, whether derived biochemically or by use of recombinant DNA. Examples include *inter alia*, F(ab), single chain Fv, and Ig variable region-phage coat protein fusions.

15 ENCODE – an open-ended term such that a nucleic acid encoding a particular amino acid sequence can consist of codons specifying that (poly)peptide, but can also comprise additional sequences either translatable, or for the control of transcription, translation, or replication, or to facilitate manipulation of some host nucleic acid construct.

20 SUBSTANTIAL SIMILARITY – this term is used to refer to sequences that differ from a reference sequence in an inconsequential way as judged by examination of the sequence. Nucleic acid sequences encoding the same amino acid sequence are substantially similar despite differences in degenerate positions or modest differences in length or composition of any non-coding regions. Amino acid sequences differing only by conservative substitution or minor length variations are substantially similar. Additionally, amino acid sequences comprising housekeeping epitopes that differ in the number of N-terminal flanking residues, or immune epitopes and epitope clusters that differ in the number of flanking residues at either terminus, are substantially similar. Nucleic acids
25 that encode substantially similar amino acid sequences are themselves also substantially similar.

30 FUNCTIONAL SIMILARITY – this term is used to refer to sequences that differ from a reference sequence in an inconsequential way as judged by examination of a biological or biochemical property, although the sequences may not be substantially similar. For example, two nucleic acids can be useful as hybridization probes for the same sequence but encode differing amino acid sequences. Two peptides that induce cross-reactive CTL responses are functionally similar even if they differ by non-conservative amino acid substitutions (and thus do not meet the substantial similarity definition). Pairs of antibodies, or TCRs, that recognize the same epitope can be functionally similar to each other despite whatever structural differences exist. In testing for functional similarity of immunogenicity one would generally immunize with the “altered” antigen
35 and test the ability of the elicited response (Ab, CTL, cytokine production, etc.) to recognize the target antigen. Accordingly, two sequences may be designed to differ in certain respects while

retaining the same function. Such designed sequence variants are among the embodiments of the present invention.

Table 1A. SEQ ID NOS.* including epitopes in Examples 1-7, 13.

SEQ ID NO	IDENTITY	SEQUENCE
1	Tyr 207-216	FLPWHRLFLL
2	Tyrosinase protein	Accession number**: P14679
3	SSX-2 protein	Accession number: NP_003138
4	PSMA protein	Accession number: NP_004467
5	Tyrosinase cDNA	Accession number: NM_000372
6	SSX-2 cDNA	Accession number: NM_003147
7	PSMA cDNA	Accession number: NM_004476
8	Tyr 207-215	FLPWHRLFL
9	Tyr 208-216	LPWHRLFLL
10	SSX-2 31-68	YFSKEEWEKMKASEKIFYVYMKRKYEAMTKLGFK ATLP
11	SSX-2 32-40	FSKEEWEKM
12	SSX-2 39-47	KMKASEKIF
13	SSX-2 40-48	MKASEKIFY
14	SSX-2 39-48	KMKASEKIFY
15	SSX-2 41-49	KASEKIFYV
16	SSX-2 40-49	MKASEKIFYV
17	SSX-2 41-50	KASEKIFYVY
18	SSX-2 42-49	ASEKIFYVY
19	SSX-2 53-61	RKYEAMTKL
20	SSX-2 52-61	KRKYEAMTKL
21	SSX-2 54-63	KYEAMTKLGF
22	SSX-2 55-63	YEAMTKLGF
23	SSX-2 56-63	EAMTKLGF
24	HBV18-27	FLPSDYFPSV
25	HLA-B44 binder	AEMGKYSFY
26	SSX-1 41-49	KYSEKISYV
27	SSX-3 41-49	KVSEKIVYV
28	SSX-4 41-49	KSSEKIVYV
29	SSX-5 41-49	KASEKIFYV
30	PSMA163-192	AFSPQGMPEGDLVYVNYARTEDFFKLERDM
31	PSMA 168-190	GMPEGDLVYVNYARTEDFFKLER
32	PSMA 169-177	MPEGDLVYV
33	PSMA 168-177	GMPEGDLVYV
34	PSMA 168-176	GMPEGDLVY
35	PSMA 167-176	QGMPEGDLVY
36	PSMA 169-176	MPEGDLVY

37	PSMA 171-179	EGDLVYVNY
38	PSMA 170-179	PEGDLVYVNY
39	PSMA 174-183	LVYVNYARTE
40	PSMA 177-185	VNYARTEDF
41	PSMA 176-185	YVNYARTEDF
42	PSMA 178-186	NYARTEDFF
43	PSMA 179-186	YARTEDFF
44	PSMA 181-189	RTEDFFKLE
45	PSMA 281-310	RGIAEAVGLPSIPVHPIGYDDAQKLEKMG
46	PSMA 283-307	IAEAVGLPSIPVHPIGYDDAQKLE
47	PSMA 289-297	LPSIPVHPI
48	PSMA 288-297	GLPSIPVHPI
49	PSMA 297-305	IGYYDAQKL
50	PSMA 296-305	PIGYDDAQKL
51	PSMA 291-299	SIPVHPIGY
52	PSMA 290-299	PSIPVHPIGY
53	PSMA 292-299	IPVHPIGY
54	PSMA 299-307	YYDAQKLE
55	PSMA454-481	SSIEGNYTLRVDCTPLMYSLVHLTKEL
56	PSMA 456-464	IEGNYTLRV
57	PSMA 455-464	SIEGNYTLRV
58	PSMA 457-464	EGNYTLRV
59	PSMA 461-469	TLRVDCTPL
60	PSMA 460-469	YTLRVDCTPL
61	PSMA 462-470	LRVDCTPLM
62	PSMA 463-471	RVDCTPLMY
63	PSMA 462-471	LRVDCTPLMY
64	PSMA653-687	FDKSNPIVLRMMNDQLMFLERAFIDPLGLPDRPFY
65	PSMA 660-681	VLRMMNDQLMFLERAFIDPLGL
66	PSMA 663-671	MMNDQLMFL
67	PSMA 662-671	RMMNDQLMFL
68	PSMA 662-670	RMMNDQLMF
69	Tyr 1-17	MLLAVLYCLLWSFQ TSA

Table 1B. SEQ ID NOS.* including epitopes in Examples 14 and 15.

SEQ ID NO	IDENTITY	SEQUENCE
70	GP100 protein	**Accession number: P40967
71	MAGE-1 protein	Accession number: P43355
72	MAGE-2 protein	Accession number: P43356
73	MAGE-3 protein	Accession number: P43357
74	NY-ESO-1 protein	Accession number: P78358
75	LAGE-1a protein	Accession number: CAA11116

76	LAGE-1b protein	Accession number: CAA11117
77	PRAME protein	Accession number: NP 006106
78	PSA protein	Accession number: P07288
79	PSCA protein	Accession number: O43653
80	GP100 cds	Accession number: U20093
81	MAGE-1 cds	Accession number: M77481
82	MAGE-2 cds	Accession number: L18920
83	MAGE-3 cds	Accession number: U03735
84	NY-ESO-1 cDNA	Accession number: U87459
85	PRAME cDNA	Accession number: NM 006115
86	PSA cDNA	Accession number: NM 001648
87	PSCA cDNA	Accession number: AF043498
88	GP100 630-638	LPHSSSHWL
89	GP100 629-638	QLPHSSSHWL
90	GP100 614-622	LIYRRRLMK
91	GP100 613-622	SLIYRRRLMK
92	GP100 615-622	IYRRRLMK
93	GP100 630-638	LPHSSSHWL
94	GP100 629-638	QLPHSSSHWL
95	MAGE-1 95-102	ESLFRAVI
96	MAGE-1 93-102	IILESLFRAVI
97	MAGE-1 93-101	IILESLFRAV
98	MAGE-1 92-101	CILESLFRAV
99	MAGE-1 92-100	CILESLFRA
100	MAGE-1 263-271	EFLWGPRAL
101	MAGE-1 264-271	FLWGPRAL
102	MAGE-1 264-273	FLWGPRALAE
103	MAGE-1 265-274	LWGPRALAET
104	MAGE-1 268-276	PRALAETSY
105	MAGE-1 267-276	GPRALAETSY
106	MAGE-1 269-277	RALAETSYV
107	MAGE-1 271-279	LAETSYVKV
108	MAGE-1 270-279	ALAETSYVKV
109	MAGE-1 272-280	AETSYVKVL
110	MAGE-1 271-280	LAETSYVKVL
111	MAGE-1 274-282	TSYVKVLEY
112	MAGE-1 273-282	ETSYVKVLEY
113	MAGE-1 278-286	KVLEYVIKV
114	MAGE-1 168-177	SYVLVTCLGL
115	MAGE-1 169-177	YVLVTCLGL
116	MAGE-1 170-177	VLVTCLGL
117	MAGE-1 240-248	TQDLVQEKY

118	MAGE-1 239-248	LTQDLVQEKY
119	MAGE-1 232-240	YGEPRKLLT
120	MAGE-1 243-251	LVQEKYLEY
121	MAGE-1 242-251	DLVQEKYLEY
122	MAGE-1 230-238	SAYGEPRKL
123	MAGE-1 278-286	KVLEYVIKV
124	MAGE-1 277-286	VKVLEYVIKV
125	MAGE-1 276-284	YVKVLEYVI
126	MAGE-1 274-282	TSYVKVLEY
127	MAGE-1 273-282	ETSYVKVLEY
128	MAGE-1 283-291	VIKVSARVR
129	MAGE-1 282-291	YVIKVSARVR
130	MAGE-2 115-122	ELVHFLLL
131	MAGE-2 113-122	MVELVHFLLL
132	MAGE-2 109-116	ISRKMVEL
133	MAGE-2 108-116	AI SRKMVEL
134	MAGE-2 107-116	AAISRKMVEL
135	MAGE-2 112-120	KMVELVHFL
136	MAGE-2 109-117	ISRKMVELV
137	MAGE-2 108-117	AI SRKMVELV
138	MAGE-2 116-124	LVHFLLLKY
139	MAGE-2 115-124	ELVHFLLLKY
140	MAGE-2 111-119	RKMVELVHF
141	MAGE-2 158-166	LQLVFGIEV
142	MAGE-2 157-166	YLQLVFGIEV
143	MAGE-2 159-167	QLVFGIEVV
144	MAGE-2 158-167	LQLVFGIEVV
145	MAGE-2 164-172	IEVVEVPPI
146	MAGE-2 163-172	GIEVVEVPPI
147	MAGE-2 162-170	FGIEVVEVV
148	MAGE-2 154-162	ASEYLQLVF
149	MAGE-2 153-162	KASEYLQLVF
150	MAGE-2 218-225	EEKIWEEL
151	MAGE-2 216-225	APEEKIWEEL
152	MAGE-2 216-223	APEEKIWE
153	MAGE-2 220-228	KIWEELSML
154	MAGE-2 219-228	EKIWEELSML
155	MAGE-2 271-278	FLWGPRAL
156	MAGE-2 271-279	FLWGPRALI
157	MAGE-2 278-286	LIETSYVKV
158	MAGE-2 277-286	ALIETSYVKV
159	MAGE-2 276-284	RALIETSYV

160	MAGE-2 279-287	LIETSYVKVL
161	MAGE-2 278-287	LIETSYVKVL
162	MAGE-3 271-278	FLWGPRL
163	MAGE-3 270-278	EFLWGPRL
164	MAGE-3 271-279	FLWGPRLV
165	MAGE-3 276-284	RALVETSYV
166	MAGE-3 272-280	LWGPRLVE
167	MAGE-3 271-280	FLWGPRLVE
168	MAGE-3 272-281	LWGPRLVET
169	NY-ESO-1 82-90	GPESRLLEF
170	NY-ESO-1 83-91	PESRLLEFY
171	NY-ESO-1 82-91	GPESRLLEFY
172	NY-ESO-1 84-92	ESRLLEFYL
173	NY-ESO-1 86-94	RLLEFYLAM
174	NY-ESO-1 88-96	LEFYLAMPF
175	NY-ESO-1 87-96	LLEFYLAMPF
176	NY-ESO-1 93-102	AMPFATPMEA
177	NY-ESO-1 94-102	MPFATPMEA
178	NY-ESO-1 115-123	PLPVPGVLL
179	NY-ESO-1 114-123	PPLPVPGVLL
180	NY-ESO-1 116-123	LPVPGVLL
181	NY-ESO-1 103-112	ELARRSLAQD
182	NY-ESO-1 118-126	VPGVLLKEF
183	NY-ESO-1 117-126	PVPGVLLKEF
184	NY-ESO-1 116-123	LPVPGVLL
185	NY-ESO-1 127-135	TVSGNILTI
186	NY-ESO-1 126-135	FTVSGNILTI
187	NY-ESO-1 120-128	GVLLKEFTV
188	NY-ESO-1 121-130	VLLKEFTVSG
189	NY-ESO-1 122-130	LLKEFTVSG
190	NY-ESO-1 118-126	VPGVLLKEF
191	NY-ESO-1 117-126	PVPGVLLKEF
192	NY-ESO-1 139-147	AADHRQLQL
193	NY-ESO-1 148-156	SISSCLQQL
194	NY-ESO-1 147-156	LSISSCLQQL
195	NY-ESO-1 138-147	TAADHRQLQL
196	NY-ESO-1 161-169	WITQCFLPV
197	NY-ESO-1 157-165	SLLMWITQC
198	NY-ESO-1 150-158	SSCLQQLSL
199	NY-ESO-1 154-162	QQLSLLMWI
200	NY-ESO-1 151-159	SCLQQLSLL
201	NY-ESO-1 150-159	SSCLQQLSLL

202	NY-ESO-1 163-171	TQCFLPVFL
203	NY-ESO-1 162-171	ITQCFLPVFL
204	PRAME 219-227	PMQDIKMIL
205	PRAME 218-227	MPMQDIKMIL
206	PRAME 428-436	QHLIGLSNL
207	PRAME 427-436	LQHLIGLSNL
208	PRAME 429-436	HLIGLSNL
209	PRAME 431-439	IGLSNLTHV
210	PRAME 430-439	LIGLSNLTHV
211	PSA 53-61	VLVHPQWVL
212	PSA 52-61	GVLVHPQWVL
213	PSA 52-60	GVLVHPQWV
214	PSA 59-67	WVLTAAHCI
215	PSA 54-63	LVHPQWVLT
216	PSA 53-62	VLVHPQWVLT
217	PSA 54-62	LVHPQWVLT
218	PSA 66-73	CIRNKSVI
219	PSA 65-73	HCIRNKSVI
220	PSA 56-64	HPQWVLTAA
221	PSA 63-72	AAHCIRNKSV
222	PSCA 116-123	LLWGPGQL
223	PSCA 115-123	LLLWGPGQL
224	PSCA 114-123	GLLLWGPGQL
225	PSCA 99-107	ALQPAAAIL
226	PSCA 98-107	HALQPAAAIL
227	Tyr 128-137	APEKDKFFAY
228	Tyr 129-137	PEKDKFFAY
229	Tyr 130-138	EKDKFFAYL
230	Tyr 131-138	KDKFFAYL
231	Tyr 205-213	PAFLPWHRL
232	Tyr 204-213	APAFLPWHRL
233	Tyr 214-223	FLLRWEQEIQ
234	Tyr 212-220	RLFLLRWEQ
235	Tyr 191-200	GSEIWRDIDF
236	Tyr 192-200	SEIWRDIDF
237	Tyr 473-481	RIWSWLLGA
238	Tyr 476-484	SWLLGAAMV
239	Tyr 477-486	WLLGAAMVGA
240	Tyr 478-486	LLGAAMVGA
241	PSMA 4-12	LLHETDSAV
242	PSMA 13-21	ATARRPRWL
243	PSMA 53-61	TPKHNMKAF

244	PSMA 64-73	ELKAENIKKF
245	PSMA 69-77	NIKKFLH ¹ NF
246	PSMA 68-77	ENIKKFLH ¹ NF
247	PSMA 220-228	AGAKGVILY
248	PSMA 468-477	PLMYSLVHNL
249	PSMA 469-477	LMYSLVHNL
250	PSMA 463-471	RVDCTPLMY
251	PSMA 465-473	DCTPLMYSL
252	PSMA 507-515	SGMPRISKL
253	PSMA 506-515	FSGMPRISKL
254	NY-ESO-1 136-163	RLTAADHRQLQLSISSCLQQLSLLMWIT
255	NY-ESO-1 150-177	SSCLQQLSLLMWITQCFLPVFLAQPPSG

¹This H was reported as Y in the SWISSPROT database.

Table 1C. SEQ ID NOS.* including epitopes in Example14.

SEQ ID NO.	IDENTITY	SEQUENCE
256	Mage-1 125-132	KAEMLESV
257	Mage-1 124-132	TKAEMLESV
258	Mage-1 123-132	VTKAEMLESV
259	Mage-1 128-136	MLESVIKNY
260	Mage-1 127-136	EMLESVIKNY
261	Mage-1 125-133	KAEMLESVI
262	Mage-1 146-153	KASESLQL
263	Mage-1 145-153	GKASESLQL
264	Mage-1 147-155	ASESLQLVF
265	Mage-1 153-161	LVFGIDVKE
266	Mage-1 114-121	LLKYRARE
267	Mage-1 106-113	VADLVGFL
268	Mage-1 105-113	KVADLVGFL
269	Mage-1 107-115	ADLVGFLL
270	Mage-1 106-115	VADLVGFLL
271	Mage-1 114-123	LLKYRAREPV
272	Mage-3 278-286	LVETSYVKV
273	Mage-3 277-286	ALVETSYVKV
274	Mage-3 285-293	KVLHHMVKI
275	Mage-3 283-291	YVKVLHHMV
276	Mage-3 275-283	PRALVETSY
277	Mage-3 274-283	GPRALVETSY
278	Mage-3 278-287	LVETSYVKVL
279	ED-B 4'-5	TIPEVPQL
280	ED-B 5'-5	DTIPEVPQL
281	ED-B 1-10	EVPLTDLSF
282	ED-B 23-30	TPLNSSTI
283	ED-B 18-25	IGLRWTPL
284	ED-B 17-25	SIGLRWTPL
285	ED-B 25-33	LNSSTIGY
286	ED-B 24-33	PLNSSTIGY

287	ED-B 23-31	TPLNSTII
288	ED-B 31-38	IGYRITVV
289	ED-B 30-38	IIGYRITVV
290	ED-B 29-38	TIIGYRITVV
291	ED-B 31-39	IGYRITVVA
292	ED-B 30-39	IIGYRITVVA
293	CEA 184-191	SLPVSPRL
294	CEA 183-191	QSLPVSPRL
295	CEA 186-193	PVSPRLQL
296	CEA 185-193	LPVSPRLQL
297	CEA 184-193	SLPVSPRLQL
298	CEA 185-192	LPVSPRLQ
299	CEA 192-200	QLSNGNRTL
300	CEA 191-200	LQLSNGNRTL
301	CEA 179-187	WVNNQSLPV
302	CEA 186-194	PVSPRLQLS
303	CEA 362-369	SLPVSPRL
304	CEA 361-369	QSLPVSPRL
305	CEA 364-371	PVSPRLQL
306	CEA 363-371	LPVSPRLQL
307	CEA 362-371	SLPVSPRLQL
308	CEA 363-370	LPVSPRLQ
309	CEA 370-378	QLSNDNRTL
310	CEA 369-378	LQLSNDNRTL
311	CEA 357-365	WVNNQSLPV
312	CEA 360-368	NQSLPVSPR
313	CEA 540-547	SLPVSPRL
314	CEA 539-547	QSLPVSPRL
315	CEA 542-549	PVSPRLQL
316	CEA 541-549	LPVSPRLQL
317	CEA 540-549	SLPVSPRLQL
318	CEA 541-548	LPVSPRLQ
319	CEA 548-556	QLSNGNRTL
320	CEA 547-556	LQLSNGNRTL
321	CEA 535-543	WVNGQSLPV
322	CEA 533-541	LWWVNGQSL
323	CEA 532-541	YLWWVNGQSL
324	CEA 538-546	GQSLPVSPR
325	Her-2 30-37	DMKLRLPA
326	Her-2 28-37	GTDMKLRLPA
327	Her-2 42-49	HLDMLRHL
328	Her-2 41-49	THLDMLRHL
329	Her-2 40-49	ETHLDMLRHL
330	Her-2 36-43	PASPETHL
331	Her-2 35-43	LPASPETHL
332	Her-2 34-43	RLPASPETHL
333	Her-2 38-46	SPETHDML
334	Her-2 37-46	ASPETHDML
335	Her-2 42-50	HLDMLRHLY
336	Her-2 41-50	THLDMLRHLY
337	Her-2 719-726	ELRKVKVL

338	Her-2 718-726	TELKVKVL
339	Her-2 717-726	ETELKVKVL
340	Her-2 715-723	LKETELRKV
341	Her-2 714-723	ILKETELRKV
342	Her-2 712-720	MRILKETEL
343	Her-2 711-720	QMRILKETEL
344	Her-2 717-725	ETELKVKV
345	Her-2 716-725	KETELKVKV
346	Her-2 706-714	MPNQQMRI
347	Her-2 705-714	AMPNQQMRI
348	Her-2 706-715	MPNQQMRL
349	HER-2 966-973	RPRFREL
350	HER-2 965-973	CRPRFREL
351	HER-2 968-976	RFRELSEF
352	HER-2 967-976	PRFRELSEF
353	HER-2 964-972	ECRPRFREL
354	NY-ESO-1 67-75	GAASGLNGC
355	NY-ESO-1 52-60	RASGPGGGA
356	NY-ESO-1 64-72	PHGGAASGL
357	NY-ESO-1 63-72	GPHGGAASGL
358	NY-ESO-1 60-69	APRGPHGGAA
359	PRAME 112-119	VRPRRWKL
360	PRAME 111-119	EVRPRRWKL
361	PRAME 113-121	RPRRWKLQV
362	PRAME 114-122	PRRWKLQVL
363	PRAME 113-122	RPRRWKLQVL
364	PRAME 116-124	RWKLQVLDL
365	PRAME 115-124	RRWKLQVLDL
366	PRAME 174-182	PVEVLVDF
367	PRAME 199-206	VKRKKNVL
368	PRAME 198-206	KVKRKKNVL
369	PRAME 197-206	EKKRKKNVL
370	PRAME 198-205	KVKRKKNV
371	PRAME 201-208	RKKNVRL
372	PRAME 200-208	KRKKNVRL
373	PRAME 199-208	VKRKKNVRL
374	PRAME 189-196	DEFSYLI
375	PRAME 205-213	VLRCCCKKL
376	PRAME 204-213	NVLRCCCKKL
377	PRAME 194-202	YLIEKVKRK
378	PRAME 74-81	QAWPFTCL
379	PRAME 73-81	VQAWPFTCL
380	PRAME 72-81	MVQAWPFTCL
381	PRAME 81-88	LPLGVLMK
382	PRAME 80-88	CLPLGVLMK
383	PRAME 79-88	TCLPLGVLMK
384	PRAME 84-92	GVLMKGQHL
385	PRAME 81-89	LPLGVLMKG
386	PRAME 80-89	CLPLGVLMKG
387	PRAME 76-85	WPFTCLPLGV
388	PRAME 51-59	ELFPPLFMA

389	PRAME 49-57	PRELFPPLF
390	PRAME 48-57	LPRELFPPLF
391	PRAME 50-58	RELFPPLFM
392	PRAME 49-58	PRELFPPLFM
393	PSA 239-246	RPSLYTKV
394	PSA 238-246	ERPSLYTKV
395	PSA 236-243	LPERPSLY
396	PSA 235-243	ALPERPSLY
397	PSA 241-249	SLYTKVVHY
398	PSA 240-249	PSLYTKVVHY
399	PSA 239-247	RPSLYTKVV
400	PSMA 211-218	GNKVKNAQ
401	PSMA 202-209	IARYGKVF
402	PSMA 217-225	AQLAGAKGV
403	PSMA 207-215	KVFRGNKVK
404	PSMA 211-219	GNKVKNAQL
405	PSMA 269-277	TPGYPANAY
406	PSMA 268-277	LTPGYPANAY
407	PSMA 271-279	GYPANAYAY
408	PSMA 270-279	PGYPANAYAY
409	PSMA 266-274	DPLTPGYPA
410	PSMA 492-500	SLYESWTKK
411	PSMA 491-500	KSLYESWTKK
412	PSMA 486-494	EGFEGKSLY
413	PSMA 485-494	DEGFEGKSLY
414	PSMA 498-506	TKKSPSPEF
415	PSMA 497-506	WTKKSPSPEF
416	PSMA 492-501	SLYESWTKKS
417	PSMA 725-732	WGEVKRQI
418	PSMA 724-732	AWGEVKRQI
419	PSMA 723-732	KAWGEVKRQI
420	PSMA 723-730	KAWGEVKR
421	PSMA 722-730	SKAWGEVKR
422	PSMA 731-739	QIYVAAFTV
423	PSMA 733-741	YVAAFTVQA
424	PSMA 725-733	WGEVKRQIY
425	PSMA 727-735	EVKRQIYVA
426	PSMA 738-746	TVQAAAETL
427	PSMA 737-746	FTVQAAAETL
428	PSMA 729-737	KRQIYVAAF
429	PSMA 721-729	PSKAWGEVK
430	PSMA 723-731	KAWGEVKRO
431	PSMA 100-108	WKEFGLDSV
432	PSMA 99-108	QWKEFGLDSV
433	PSMA 102-111	BFGLDSVELA
434	SCP-1 126-134	ELRQKESKL
435	SCP-1 125-134	ABLQKESKL
436	SCP-1 133-141	KLQENRKII
437	SCP-1 298-305	QLEEKTKL
438	SCP-1 297-305	NQLEEKTKL
439	SCP-1 288-296	LLEESRDKV

440	SCP-1 287-296	FLLEESRDKV
441	SCP-1 291-299	ESRDKVNQL
442	SCP-1 290-299	EESRDKVNQL
443	SCP-1 475-483	EKEVHDLEY
444	SCP-1 474-483	REKEVHDLEY
445	SCP-1 480-488	DLEYSYCHY
446	SCP-1 477-485	EVHDLEYSY
447	SCP-1 477-486	EVHDLEYSYC
448	SCP-1 502-509	KLSSKREL
449	SCP-1 508-515	ELKNTEYF
450	SCP-1 507-515	RELKNTEYF
451	SCP-1 496-503	KRGORPKL
452	SCP-1 494-503	LPKRGORPKL
453	SCP-1 509-517	LKNTEYFTL
454	SCP-1 508-517	ELKNTEYFTL
455	SCP-1 506-514	KRELKNTEY
456	SCP-1 502-510	KLSSKRELK
457	SCP-1 498-506	GORPKLSSK
458	SCP-1 497-506	RGORPKLSSK
459	SCP-1 500-508	RPKLSSKRE
460	SCP-1 573-580	LEYVREEL
461	SCP-1 572-580	ELEYVREEL
462	SCP-1 571-580	NELEYVREEL
463	SCP-1 579-587	ELKQKREDEV
464	SCP-1 575-583	YVREELKQK
465	SCP-1 632-640	QLNVYEIKV
466	SCP-1 630-638	SKQLNVYEI
467	SCP-1 628-636	AESKQLNVY
468	SCP-1 627-636	TAESKQLNVY
469	SCP-1 638-645	IKVKNLEL
470	SCP-1 637-645	EIKVKNLEL
471	SCP-1 636-645	YEIKVKNLEL
472	SCP-1 642-650	KLELELESA
473	SCP-1 635-643	VYEIKVKNL
474	SCP-1 634-643	NVYEIKVKNL
475	SCP-1 646-654	ELESAKQKF
476	SCP-1 642-650	KLELELESA
477	SCP-1 646-654	ELESAKQKF
478	SCP-1 771-778	KEKCLKREA
479	SCP-1 777-785	EAKENTATL
480	SCP-1 776-785	REAKENTATL
481	SCP-1 773-782	KLKREAKENT
482	SCP-1 112-119	EAEKIKKW
483	SCP-1 101-109	GLSRVYSKL
484	SCP-1 100-109	EGLSRVYSKL
485	SCP-1 108-116	KLYKEAEKI
486	SCP-1 98-106	NSEGLSRVY
487	SCP-1 97-106	ENSEGLSRVY
488	SCP-1 102-110	LSRVYSKLY
489	SCP-1 101-110	GLSRVYSKLY
490	SCP-1 96-105	LENSEGLSRV

491	SCP-1 108-117	KLYKEAEKIK
492	SCP-1 949-956	REDRWAVI
493	SCP-1 948-956	MRREDRWAVI
494	SCP-1 947-956	KMREDRWAVI
495	SCP-1 947-955	KMREDRWAV
496	SCP-1 934-942	TTPGSTLKF
497	SCP-1 933-942	LTPGSTLKF
498	SCP-1 937-945	GSTLKGAI
499	SCP-1 945-953	IRKMREDRW
500	SCP-1 236-243	RLEMHFKL
501	SCP-1 235-243	SRLEMHFKL
502	SCP-1 242-250	KLKEDYEKI
503	SCP-1 249-257	KIQHLEQEY
504	SCP-1 248-257	EKIQHLEQEY
505	SCP-1 233-242	ENSRLEMHF
506	SCP-1 236-245	RLEMHFKLKE
507	SCP-1 324-331	LEDIKVSL
508	SCP-1 323-331	ELEDIKVSL
509	SCP-1 322-331	KELEDIKVSL
510	SCP-1 320-327	LTKELEDI
511	SCP-1 319-327	HLTKELEDI
512	SCP-1 330-338	SLQRSVSTQ
513	SCP-1 321-329	TKELEDIKV
514	SCP-1 320-329	LTKELEDIKV
515	SCP-1 326-335	DIKVSLQRSV
516	SCP-1 281-288	KMKDLTFL
517	SCP-1 280-288	NKMKDLTFL
518	SCP-1 279-288	ENKMKDLTFL
519	SCP-1 288-296	LLEESRDKV
520	SCP-1 287-296	FLLEESRDKV
521	SCP-1 291-299	ESRDKVNQL
522	SCP-1 290-299	EESRDKVNQL
523	SCP-1 277-285	EKENKMKDL
524	SCP-1 276-285	TEKENKMKDL
525	SCP-1 279-287	ENKMKDLTF
526	SCP-1 218-225	IEKMITAF
527	SCP-1 217-225	NIEKMITAF
528	SCP-1 216-225	SNIEKMITAF
529	SCP-1 223-230	TAFEELRV
530	SCP-1 222-230	ITAFEELRV
531	SCP-1 221-230	MITAFEELRV
532	SCP-1 220-228	KMITAFEEL
533	SCP-1 219-228	EKMITAFEEL
534	SCP-1 227-235	ELRVQAENS
535	SCP-1 213-222	DLNSNIEKMI
536	SCP-1 837-844	WTSAKNTL
537	SCP-1 846-854	TPLPKAYTV
538	SCP-1 845-854	STPLPKAYTV
539	SCP-1 844-852	LSTPLPKAY
540	SCP-1 843-852	TLSTPLPKAY
541	SCP-1 842-850	NTLSTPLPK

542	SCP-1 841-850	KNTLSTPLPK
543	SCP-1 828-835	ISKDKRDY
544	SCP-1 826-835	HGISKDKRDY
545	SCP-1 832-840	KRDYLWTSa
546	SCP-1 829-838	SKDKRDYLWT
547	SCP-1 279-286	ENKMKDLT
548	SCP-1 260-268	EINDKEKQV
549	SCP-1 274-282	QITEKENKM
550	SCP-1 269-277	SLLLIQITE
551	SCP-1 453-460	FEKIAEEL
552	SCP-1 452-460	QFEKIAEEL
553	SCP-1 451-460	KQFEKIAEEL
554	SCP-1 449-456	DNKQFEKI
555	SCP-1 448-456	YDNKQFEKI
556	SCP-1 447-456	LYDNKQFEKI
557	SCP-1 440-447	LGEKETLL
558	SCP-1 439-447	VLGEKETLL
559	SCP-1 438-447	KVLGEKETLL
560	SCP-1 390-398	LLRTEQQRL
561	SCP-1 389-398	ELLRTEQQRL
562	SCP-1 393-401	TEQQRLeny
563	SCP-1 392-401	RTEQQRLeny
564	SCP-1 402-410	EDQLILTM
565	SCP-1 397-406	RLENYEDQLI
566	SCP-1 368-375	KARAAHSF
567	SCP-1 376-384	VVTEFETTV
568	SCP-1 375-384	FVVTEFETTV
569	SCP-1 377-385	VTEFETTVc
570	SCP-1 376-385	VVTEFETTVc
571	SCP-1 344-352	DLQIATNTI
572	SCP-1 347-355	IATNTICQL
573	SCP-1 346-355	QIATNTICQL
574	SSX4 57-65	VMTKLGFKY
575	SSX4 53-61	LNYEVMTKL
576	SSX4 52-61	KLNYEVMTKL
577	SSX4 66-74	TLPPFMRSK
578	SSX4 110-118	KIMPKKPAE
579	SSX4 103-112	SLQRIFPKIM
580	Tyr 463-471	YIKSYLEQA
581	Tyr 459-467	SFQDYIKSY
582	Tyr 458-467	DSFQDYIKSY
583	Tyr 507-514	LPEEKQPL
584	Tyr 506-514	QLPEEKQPL
585	Tyr 505-514	KQLPEEKQPL
586	Tyr 507-515	LPEEKQPLL
587	Tyr 506-515	QLPEEKQPLL
588	Tyr 497-505	SLLCRHKRK
589	ED-B domain of Fibronectin	EVpQLTDLSFVDITDSSIGLRWTPLNSSTiGYRI TVVAAGEGIPIFEDFVDSSVGYYTvtGLEPGID YDISVITLINGGESAPTTLTQQT
590	ED-B domain of	CTFDNLSPGLEYNVSVYTVKDDKESVPISDTIP

	Fibronectin with flanking sequence from Fribronectin	EVPQLTDLSTFVDITDSSIGLRWTPPLNSSTIIGYRI TVVAAGEGIPFEDFVDSSVGYTFTGLEPGID YDISVITLINGGESAPTTLTQQT AVPPPTDLRFTNIGPDTMRVTW
591	ED-B domain of Fibronectin cds	Accession number: X07717
592	CEA protein	Accession number: P06731
593	CEA cDNA	Accession number: NM_004363
594	Her2/Neu protein	Accession number: P04626
595	Her2/Neu cDNA	Accession number: M11730
596	SCP-1 protein	Accession number: Q15431
597	SCP-1 cDNA	Accession number: X95654
598	SSX-4 protein	Accession number: O60224
599	SSX-4 cDNA	Accession number: NM_005636

*Any of SEQ ID NOS. 1, 8, 9, 11-23, 26-29, 32-44, 47-54, 56-63, 66-68 88-253, and 256-588 can be useful as epitopes in any of the various embodiments of the invention. Any of SEQ ID NOS. 10, 30, 31, 45, 46, 55, 64, 65, 69, 254, and 255 can be useful as sequences containing epitopes or epitope clusters, as described in various embodiments of the invention.

**All accession numbers used here and throughout can be accessed through the NCBI databases, for example, through the Entrez seek and retrieval system on the world wide web.

Note that the following discussion sets forth the inventors' understanding of the operation of the invention. However, it is not intended that this discussion limit the patent to any particular theory of operation not set forth in the claims.

In pursuing the development of epitope vaccines others have generated lists of predicted epitopes based on MHC binding motifs. Such peptides can be immunogenic, but may not correspond to any naturally produced antigenic fragment so that whole antigen will not elicit a similar response or sensitize a target cell to cytolysis by CTL. Therefore such lists do not differentiate between those sequences that can be useful as vaccines and those that cannot. Efforts to determine which of these predicted epitopes are in fact naturally produced have often relied on screening their reactivity with tumor infiltrating lymphocytes (TIL). However, TIL are strongly biased to recognize immune epitopes whereas tumors (and chronically infected cells) will generally present housekeeping epitopes. Thus, unless the epitope is produced by both the housekeeping and immuno- proteasomes, the target cell will generally not be recognized by CTL induced with TIL-identified epitopes. The epitopes of the present invention, in contrast, are generated by the action a specified proteasome, indicating that they can be naturally produced, and enabling their appropriate use. The importance of the distinction between housekeeping and immune epitopes to vaccine design in more fully set forth in PCT publication WO 01/82963A2.

The epitopes of the invention include or encode polypeptide fragments of TAAs that are precursors or products of proteasomal cleavage by a housekeeping or immune proteasome, and that have known or predicted affinity for at least one allele of MHC I. In some embodiments, the epitopes include or encode a polypeptide of about 6 to 25 amino acids in length, preferably about 7

to 20 amino acids in length, more preferably about 8 to 15 amino acids in length, and still more preferably 9 or 10 amino acids in length. However, it is understood that the polypeptides can be larger as long as they do not contain sequences that cause the polypeptides to be directed away from the proteasome or to be destroyed by the proteasome. For immune epitopes, if the larger peptides do not contain such sequences, they can be processed in the pAPC by the immune proteasome. Housekeeping epitopes may also be embedded in longer sequences provided that the sequence is adapted to facilitate liberation of the epitope's C-terminus by action of the immunoproteasome. The sequences of these epitopes can be subjected to computer analysis in order to calculate physical, biochemical, immunologic, or molecular genetic properties such as mass, isoelectric point, predicted mobility in electrophoresis, predicted binding to other MHC molecules, melting temperature of nucleic acid probes, reverse translations, similarity or homology to other sequences, and the like.

In constructing the polynucleotides encoding the polypeptide epitopes of the invention, the gene sequence of the associated TAA can be used, or the polynucleotide can be assembled from any of the corresponding codons. For a 10 amino acid epitope this can constitute on the order of 10^6 different sequences, depending on the particular amino acid composition. While large, this is a distinct and readily definable set representing a miniscule fraction of the $>10^{18}$ possible polynucleotides of this length, and thus in some embodiments, equivalents of a particular sequence disclosed herein encompass such distinct and readily definable variations on the listed sequence. In choosing a particular one of these sequences to use in a vaccine, considerations such as codon usage, self-complementarity, restriction sites, chemical stability, etc. can be used as will be apparent to one skilled in the art.

The invention contemplates producing peptide epitopes. Specifically these epitopes are derived from the sequence of a TAA, and have known or predicted affinity for at least one allele of MHC I. Such epitopes are typically identical to those produced on target cells or pAPCs.

Compositions Containing Active Epitopes

Embodiments of the present invention provide polypeptide compositions, including vaccines, therapeutics, diagnostics, pharmacological and pharmaceutical compositions. The various compositions include newly identified epitopes of TAAs, as well as variants of these epitopes. Other embodiments of the invention provide polynucleotides encoding the polypeptide epitopes of the invention. The invention further provides vectors for expression of the polypeptide epitopes for purification. In addition, the invention provides vectors for the expression of the polypeptide epitopes in an APC for use as an anti-tumor vaccine. Any of the epitopes or antigens, or nucleic acids encoding the same, from Table 1A can be used. Other embodiments relate to methods of making and using the various compositions.

A general architecture for a class I MHC-binding epitope can be described, and has been reviewed more extensively in Madden, D.R. *Annu. Rev. Immunol.* 13:587-622, 1995. Much of the binding energy arises from main chain contacts between conserved residues in the MHC molecule and the N- and C-termini of the peptide. Additional main chain contacts are made but vary among MHC alleles. Sequence specificity is conferred by side chain contacts of so-called anchor residues with pockets that, again, vary among MHC alleles. Anchor residues can be divided into primary and secondary. Primary anchor positions exhibit strong preferences for relatively well-defined sets of amino acid residues. Secondary positions show weaker and/or less well-defined preferences that can often be better described in terms of less favored, rather than more favored, residues. Additionally, residues in some secondary anchor positions are not always positioned to contact the pocket on the MHC molecule at all. Thus, a subset of peptides exists that bind to a particular MHC molecule and have a side chain-pocket contact at the position in question and another subset exists that show binding to the same MHC molecule that does not depend on the conformation the peptide assumes in the peptide-binding groove of the MHC molecule. The C-terminal residue (P₋₁) is preferably a primary anchor residue. For many of the better studied HLA molecules (e.g. A2, A68, B27, B7, B35, and B53) the second position (P2) is also an anchor residue. However, central anchor residues have also been observed including P3 and P5 in HLA-B8, as well as P5 and P₋₃ in the murine MHC molecules H-2D^b and H-2K^b, respectively. Since more stable binding will generally improve immunogenicity, anchor residues are preferably conserved or optimized in the design of variants, regardless of their position.

Because the anchor residues are generally located near the ends of the epitope, the peptide can buckle upward out of the peptide-binding groove allowing some variation in length. Epitopes ranging from 8-11 amino acids have been found for HLA-A68, and up to 13 amino acids for HLA-A2. In addition to length variation between the anchor positions, single residue truncations and extensions have been reported and the N- and C-termini, respectively. Of the non-anchor residues, some point up out of the groove, making no contact with the MHC molecule but being available to contact the TCR, very often P1, P4, and P₋₁ for HLA-A2. Others of the non-anchor residues can become interposed between the upper edges of the peptide-binding groove and the TCR, contacting both. The exact positioning of these side chain residues, and thus their effects on binding, MHC fine conformation, and ultimately immunogenicity, are highly sequence dependent. For an epitope to be highly immunogenic it must not only promote stable enough TCR binding for activation to occur, but the TCR must also have a high enough off-rate that multiple TCR molecules can interact sequentially with the same peptide-MHC complex (Kalergis, A.M. et al., *Nature Immunol.* 2:229-234, 2001). Thus without further information about the ternary complex, both conservative and non-conservative substitutions at these positions merit consideration when designing variants.

The polypeptide epitope variants can be made, for example, using any of the techniques and guidelines for conservative and non-conservative mutations. Variants can be derived from substitution, deletion or insertion of one or more amino acids as compared with the native sequence. Amino acid substitutions can be the result of replacing one amino acid with another amino acid having similar structural and/or chemical properties, such as the replacement of a threonine with a serine. Such replacements are referred to as conservative amino acid replacements, and all appropriate conservative amino acid replacements are considered to be embodiments of one invention. Insertions or deletions can optionally be in the range of about 1 to 4, preferably 1 to 2, amino acids. It is generally preferable to maintain the "anchor positions" of the peptide which are responsible for binding to the MHC molecule in question. Indeed, immunogenicity of peptides can be improved in many cases by substituting more preferred residues at the anchor positions (Franco, et al., *Nature Immunology*, 1(2):145-150, 2000). Immunogenicity of a peptide can also often be improved by substituting bulkier amino acids for small amino acids found in non-anchor positions while maintaining sufficient cross-reactivity with the original epitope to constitute a useful vaccine. The variation allowed can be determined by routine insertions, deletions or substitutions of amino acids in the sequence and testing the resulting variants for activity exhibited by the polypeptide epitope. Because the polypeptide epitope is often 9 amino acids, the substitutions preferably are made to the shortest active epitope, for example, an epitope of 9 amino acids.

Variants can also be made by adding any sequence onto the N-terminus of the polypeptide epitope variant. Such N-terminal additions can be from 1 amino acid up to at least 25 amino acids. Because peptide epitopes are often trimmed by N-terminal exopeptidases active in the pAPC, it is understood that variations in the added sequence can have no effect on the activity of the epitope. In preferred embodiments, the amino acid residues between the last upstream proteasomal cleavage site and the N-terminus of the MHC epitope do not include a proline residue. Serwold, T. et al., *Nature Immunol.* 2:644-651, 2001. Accordingly, effective epitopes can be generated from precursors larger than the preferred 9-mer class I motif.

Peptides are useful to the extent that they correspond to epitopes actually displayed by MHC I on the surface of a target cell or a pACP. A single peptide can have varying affinities for different MHC molecules, binding some well, others adequately, and still others not appreciably (Table 2). MHC alleles have traditionally been grouped according to serologic reactivity which does not reflect the structure of the peptide-binding groove, which can differ among different alleles of the same type. Similarly, binding properties can be shared across types; groups based on shared binding properties have been termed supertypes. There are numerous alleles of MHC I in the human population; epitopes specific to certain alleles can be selected based on the genotype of the patient.

Table 2.
Predicted Binding of Tyrosinase₂₀₇₋₂₁₆ (SEQ ID NO. 1) to Various MHC types

MHC I type	*Half time of dissociation (min)
A1	0.05
A*0201	1311.
A*0205	50.4
A3	2.7
A*1101 (part of the A3 supertype)	0.012
A24	6.0
B7	4.0
B8	8.0
B14 (part of the B27 supertype)	60.0
B*2702	0.9
B*2705	30.0
B*3501 (part of the B7 supertype)	2.0
B*4403	0.1
B*5101 (part of the B7 supertype)	26.0
B*5102	55.0
B*5801	0.20
B60	0.40
B62	2.0

*HLA Peptide Binding Predictions (internet http://bimas.dcrt.nih.gov/molbio/hla_bin)

5 In further embodiments of the invention, the epitope, as peptide or encoding polynucleotide, can be administered as a vaccine or immunogenic composition, alone or in combination with various adjuvants, carriers, or excipients. It should be noted that although the term vaccine may be used herein, the discussion can be applied and used with any of the other compositions mentioned herein. Particularly advantageous adjuvants include various cytokines and oligonucleotides containing immunostimulatory sequences (as set forth in greater detail in the co-pending applications referenced herein). Additionally the polynucleotide encoded epitope can be contained in a virus (e.g. *vaccinia* or adenovirus) or in a microbial host cell (e.g. *Salmonella* or *Listeria monocytogenes*) which is then used as a vector for the polynucleotide (Dietrich, G. et al. Nat. Biotech. 16:181-185, 1998). Alternatively a pAPC can be transformed, *ex vivo*, to express the epitope, or pulsed with peptide epitope, to be itself administered as a vaccine. To increase efficiency of these processes, the encoded epitope can be carried by a viral or bacterial vector, or complexed with a ligand of a receptor found on pAPC. Similarly the peptide epitope can be complexed with or conjugated to a pAPC ligand. A vaccine can be composed of more than a single epitope.

Particularly advantageous strategies for incorporating epitopes, and combining them with epitope clusters, into a vaccine are disclosed in U.S. Patent Application No. 09/560,465 entitled "EPIOTOPE SYNCHRONIZATION IN ANTIGEN PRESENTING CELLS," filed on April 28,

2000. Epitope clusters for use in connection with this invention are disclosed in U.S. Patent Application No. 09/561,571 entitled "EPITOPE CLUSTERS," filed on April 28, 2000.

5 Preferred embodiments of the present invention are directed to vaccines and methods for causing a pAPC or population of pAPCs to present housekeeping epitopes that correspond to the epitopes displayed on a particular target cell. Any of the epitopes or antigens in Table 1A, can be used for example. In one embodiment, the housekeeping epitope is a TuAA epitope processed by the housekeeping proteasome of a particular tumor type. In another embodiment, the housekeeping epitope is a virus-associated epitope processed by the housekeeping proteasome of a cell infected with a virus. This facilitates a specific T cell response to the target cells. Concurrent expression
10 by the pAPCs of multiple epitopes, corresponding to different induction states (pre- and post-attack), can drive a CTL response effective against target cells as they display either housekeeping epitopes or immune epitopes.

By having both housekeeping and immune epitopes present on the pAPC, this embodiment can optimize the cytotoxic T cell response to a target cell. With dual epitope expression, the pAPCs can continue to sustain a CTL response to the immune-type epitope when the tumor cell switches from the housekeeping proteasome to the immune proteasome with induction by IFN, which, for example, may be produced by tumor-infiltrating CTLs.
15

In a preferred embodiment, immunization of a patient is with a vaccine that includes a housekeeping epitope. Many preferred TAAs are associated exclusively with a target cell, particularly in the case of infected cells. In another embodiment, many preferred TAAs are the result of deregulated gene expression in transformed cells, but are found also in tissues of the testis, ovaries and fetus. In another embodiment, useful TAAs are expressed at higher levels in the target cell than in other cells. In still other embodiments, TAAs are not differentially expressed in the target cell compare to other cells, but are still useful since they are involved in a particular function
20 of the cell and differentiate the target cell from most other peripheral cells; in such embodiments, healthy cells also displaying the TAA may be collaterally attacked by the induced T cell response, but such collateral damage is considered to be far preferable to the condition caused by the target cell.
25

A preferred embodiment of the present invention includes a method of administering a vaccine including a housekeeping epitope to induce a therapeutic immune response. The vaccine is administered to a patient in a manner consistent with the standard vaccine delivery protocols that are well known in the art. Methods of administering epitopes of TAAs include, without limitation, transdermal, intranodal, perinodal, oral, intravenous, intradermal, intramuscular, intraperitoneal, and mucosal administration. A particularly useful method of vaccine delivery to elicit a CTL
30 response is disclosed in PCT Publication No. WO 99/01283, entitled "A METHOD OF INDUCING A CTL RESPONSE," filed on July 10, 1998.
35

Because the epitope synchronization system has utility in inducing a cell mediated immune response, a vaccine to induce a specific T cell response to a target cell is likewise included in a preferred embodiment of the present invention. The vaccine contains a housekeeping epitope in a concentration effective to cause a pAPC or populations of pAPCs to display housekeeping epitopes. Advantageously, the vaccine can include a plurality of housekeeping epitopes or one or more housekeeping epitopes optionally in combination with one or more immune epitopes. Formulations of the vaccine contain peptides and/or nucleic acids in a concentration sufficient to cause pAPCs to present the epitopes. The formulations preferably contain epitopes in a total concentration of about 1µg-1mg/100µl of vaccine preparation. Conventional dosages and dosing for peptide vaccines and/or nucleic acid vaccines can be used with the present invention, and such dosing regimens are well understood in the art. In one embodiment, a single dosage for an adult human may advantageously be from about 1 to about 5000 µl of such a composition, administered one time or multiple times, e.g., in 2, 3, 4 or more dosages separated by 1 week, 2 weeks, 1 month, or more. insulin pump delivers 1 ul per hour (lowest frequency) ref intranodal method patent.

The compositions and methods of the invention disclosed herein further contemplate incorporating adjuvants into the formulations in order to enhance the performance of the vaccines. Specifically, the addition of adjuvants to the formulations is designed to enhance the delivery or uptake of the epitopes by the pAPCs. The adjuvants contemplated by the present invention are known by those of skill in the art and include, for example, GMCSF, GCSF, IL-2, IL-12, BCG, tetanus toxoid, osteopontin, and ETA-1.

In some embodiments of the invention, the vaccines can include a recombinant organism, such as a virus, bacterium or parasite, genetically engineered to express an epitope in a host. For example, *Listeria monocytogenes*, a gram-positive, facultative intracellular bacterium, is a potent vector for targeting TuAAs to the immune system. In a preferred embodiment, this vector can be engineered to express a housekeeping epitope to induce therapeutic responses. The normal route of infection of this organism is through the gut and can be delivered orally. In another embodiment, an adenovirus (Ad) vector encoding a housekeeping epitope for a TuAA can be used to induce anti-virus or anti-tumor responses. Bone marrow-derived dendritic cells can be transduced with the virus construct and then injected, or the virus can be delivered directly via subcutaneous injection into an animal to induce potent T-cell responses. Another embodiment employs a recombinant vaccinia virus engineered to encode amino acid sequences corresponding to a housekeeping epitope for a TAA. Vaccinia viruses carrying constructs with the appropriate nucleotide substitutions in the form of a minigene construct can direct the expression of a housekeeping epitope, leading to a therapeutic T cell response against the epitope.

The immunization with DNA requires that APCs take up the DNA and express the encoded proteins or peptides. It is possible to encode a discrete class I peptide on the DNA. By

immunizing with this construct, APCs can be caused to express a housekeeping epitope, which is then displayed on class I MHC on the surface of the cell for stimulating an appropriate CTL response. Constructs generally relying on termination of translation or non-proteasomal proteases for generation of proper termini of housekeeping epitopes have been described in U.S. Patent application No. 09/561,572 entitled EXPRESSION VECTORS ENCODING EPITOPES OF
5 TARGET-ASSOCIATED ANTIGENS, filed on April 28, 2000.

It can be desirable to express housekeeping peptides in the context of a larger protein. Processing can be detected even when a small number of amino acids are present beyond the terminus of an epitope. Small peptide hormones are usually proteolytically processed from longer
10 translation products, often in the size range of approximately 60-120 amino acids. This fact has led some to assume that this is the minimum size that can be efficiently translated. In some embodiments, the housekeeping peptide can be embedded in a translation product of at least about 60 amino acids. In other embodiments the housekeeping peptide can be embedded in a translation product of at least about 50, 30, or 15 amino acids.

Due to differential proteasomal processing, the immune proteasome of the pAPC produces peptides that are different from those produced by the housekeeping proteasome in peripheral body cells. Thus, in expressing a housekeeping peptide in the context of a larger protein, it is preferably expressed in the APC in a context other than its full length native sequence, because, as a housekeeping epitope, it is generally only efficiently processed from the native protein by the
20 housekeeping proteasome, which is not active in the APC. In order to encode the housekeeping epitope in a DNA sequence encoding a larger protein, it is useful to find flanking areas on either side of the sequence encoding the epitope that permit appropriate cleavage by the immune proteasome in order to liberate that housekeeping epitope. Such a sequence ensuring epitope synchronization is referred to hereinafter as a SYNCHROTOPE™. Altering flanking amino acid
25 residues at the N-terminus and C-terminus of the desired housekeeping epitope can facilitate appropriate cleavage and generation of the housekeeping epitope in the APC. Sequences embedding housekeeping epitopes can be designed *de novo* and screened to determine which can be successfully processed by immune proteasomes to liberate housekeeping epitopes.

Alternatively, another strategy is very effective for identifying sequences allowing
30 production of housekeeping epitopes in APC. A contiguous sequence of amino acids can be generated from head to tail arrangement of one or more housekeeping epitopes. A construct expressing this sequence is used to immunize an animal, and the resulting T cell response is evaluated to determine its specificity to one or more of the epitopes in the array. By definition, these immune responses indicate housekeeping epitopes that are processed in the pAPC effectively.
35 The necessary flanking areas around this epitope are thereby defined. The use of flanking regions of about 4-6 amino acids on either side of the desired peptide can provide the necessary

information to facilitate proteasome processing of the housekeeping epitope by the immune proteasome. Therefore, a SYNCHROTOPE™ of approximately 16-22 amino acids can be inserted into, or fused to, any protein sequence effectively to result in that housekeeping epitope being produced in an APC. In alternate embodiments the whole head-to-tail array of epitopes, or just the epitopes immediately adjacent to the correctly processed housekeeping epitope can be similarly transferred from a test construct to a vaccine vector.

In a preferred embodiment, the housekeeping epitopes can be embedded between known immune epitopes, or segments of such, thereby providing an appropriate context for processing. The abutment of housekeeping and immune epitopes can generate the necessary context to enable the immune proteasome to liberate the housekeeping epitope, or a larger fragment, preferably including a correct C-terminus. It can be useful to screen constructs to verify that the desired epitope is produced. The abutment of housekeeping epitopes can generate a site cleavable by the immune proteasome. Some embodiments of the invention employ known epitopes to flank housekeeping epitopes in test substrates; in others, screening as described below are used whether the flanking regions are arbitrary sequences or mutants of the natural flanking sequence, and whether or not knowledge of proteasomal cleavage preferences are used in designing the substrates.

Cleavage at the mature N-terminus of the epitope, while advantageous, is not required, since a variety of N-terminal trimming activities exist in the cell that can generate the mature N-terminus of the epitope subsequent to proteasomal processing. It is preferred that such N-terminal extension be less than about 25 amino acids in length and it is further preferred that the extension have few or no proline residues. Preferably, in screening, consideration is given not only to cleavage at the ends of the epitope (or at least at its C-terminus), but consideration also can be given to ensure limited cleavage within the epitope.

Shotgun approaches can be used in designing test substrates and can increase the efficiency of screening. In one embodiment multiple epitopes can be assembled one after the other, with individual epitopes possibly appearing more than once. The substrate can be screened to determine which epitopes can be produced. In the case where a particular epitope is of concern a substrate can be designed in which it appears in multiple different contexts. When a single epitope appearing in more than one context is liberated from the substrate additional secondary test substrates, in which individual instances of the epitope are removed, disabled, or are unique, can be used to determine which are being liberated and truly constitute SYNCHROTOPE™s.

Several readily practicable screens exist. A preferred *in vitro* screen utilizes proteasomal digestion analysis, using purified immune proteasomes, to determine if the desired housekeeping epitope can be liberated from a synthetic peptide embodying the sequence in question. The position of the cleavages obtained can be determined by techniques such as mass spectrometry, HPLC, and

N-terminal pool sequencing; as described in greater detail in U. S. Patent Applications entitled METHOD OF EPITOPE DISCOVERY, EPITOPE SYNCHRONIZATION IN ANTIGEN PRESENTING CELLS, two Provisional U. S. Patent Applications entitled EPITOPE SEQUENCES, which are all cited and incorporated by reference above.

5 Alternatively, *in vivo* screens such as immunization or target sensitization can be employed. For immunization a nucleic acid construct capable of expressing the sequence in question is used. Harvested CTL can be tested for their ability to recognize target cells presenting the housekeeping epitope in question. Such targets cells are most readily obtained by pulsing cells expressing the appropriate MHC molecule with synthetic peptide embodying the mature
10 housekeeping epitope. Alternatively, cells known to express housekeeping proteasome and the antigen from which the housekeeping epitope is derived, either endogenously or through genetic engineering, can be used. To use target sensitization as a screen, CTL, or preferably a CTL clone, that recognizes the housekeeping epitope can be used. In this case it is the target cell that expresses the embedded housekeeping epitope (instead of the pAPC during immunization) and it must
15 express immune proteasome. Generally, the target cell can be transformed with an appropriate nucleic acid construct to confer expression of the embedded housekeeping epitope. Loading with a synthetic peptide embodying the embedded epitope using peptide loaded liposomes or a protein transfer reagent such as BIOPORTER™ (Gene Therapy Systems, San Diego, CA) represents an alternative.

20 Additional guidance on nucleic acid constructs useful as vaccines in accordance with the present invention are disclosed in U.S. Patent Application No. 09/561,572 entitled "EXPRESSION VECTORS ENCODING EPITOPES OF TARGET-ASSOCIATED ANTIGENS," filed on April 28, 2000. Further, expression vectors and methods for their design, which are useful in accordance with the present invention are disclosed in U.S. Patent Application No. 60/336,968 (attorney
25 docket number CTLIMM.022PR) entitled "EXPRESSION VECTORS ENCODING EPITOPES OF TARGET-ASSOCIATED ANTIGENS AND METHODS FOR THEIR DESIGN," filed on 11/7/2001, which is incorporated by reference in its entirety.

 A preferred embodiment of the present invention includes a method of administering a vaccine including an epitope (or epitopes) to induce a therapeutic immune response. The vaccine
30 is administered to a patient in a manner consistent with the standard vaccine delivery protocols that are known in the art. Methods of administering epitopes of TAAs including, without limitation, transdermal, intranodal, perinodal, oral, intravenous, intradermal, intramuscular, intraperitoneal, and mucosal administration, including delivery by injection, instillation or inhalation. A particularly useful method of vaccine delivery to elicit a CTL response is disclosed in Australian
35 Patent No. 739189 issued January 17, 2002; U.S. Patent Application No. 09/380,534, filed on

September 1, 1999; and a Continuation-in-Part thereof U.S. Patent Application No. 09/776,232 both entitled "A METHOD OF INDUCING A CTL RESPONSE," filed on February 2, 2001.

Reagents Recognizing Epitopes

5 In another aspect of the invention, proteins with binding specificity for the epitope and the epitope-MHC molecule complex are contemplated, as well as the isolated cells by which they can be expressed. In one set of embodiments these reagents take the form of immunoglobulins: polyclonal sera or monoclonal antibodies (mAb), methods for the generation of which are well known in the art. Generation of mAb with specificity for peptide-MHC molecule complexes is known in the art. See, for example, Aharoni et al. *Nature* 351:147-150, 1991; Andersen et al. *Proc. Natl. Acad. Sci. USA* 93:1820-1824, 1996; Dadaglio et al. *Immunity* 6:727-738, 1997; Duc et al. *Int. Immunol.* 5:427-431, 1993; Eastman et al. *Eur. J. Immunol.* 26:385-393, 1996; Engberg et al. *Immunotechnology* 4:273-278, 1999; Porgdor et al. *Immunity* 6:715-726, 1997; Puri et al. *J. Immunol.* 158:2471-2476, 1997; and Polakova, K., et al. *J. Immunol.* 165 342-348, 2000; all of which are hereby incorporated by reference in their entirety.

15 In other embodiments the compositions can be used to induce and generate, *in vivo* and *in vitro*, T-cells specific for the any of the epitopes, including those listed in Table 1A, for example. Thus, embodiments also relate to and include isolated T cells, T cell clones, T cell hybridomas, or a protein containing the T cell receptor (TCR) binding domain derived from the cloned gene, as well as a recombinant cell expressing such a protein. Such TCR derived proteins can be simply the extra-cellular domains of the TCR, or a fusion with portions of another protein to confer a desired property or function. One example of such a fusion is the attachment of TCR binding domains to the constant regions of an antibody molecule so as to create a divalent molecule. The construction and activity of molecules following this general pattern have been reported, for example, Plaksin, D. et al. *J. Immunol.* 158:2218-2227, 1997 and Lebowitz, M.S. et al. *Cell Immunol.* 192:175-184, 20 1999, which are hereby incorporated by reference in their entirety. The more general construction and use of such molecules is also treated in U.S. patent 5,830,755 entitled T CELL RECEPTORS AND THEIR USE IN THERAPEUTIC AND DIAGNOSTIC METHODS, which is hereby incorporated by reference in its entirety.

25 The generation of such T cells can be readily accomplished by standard immunization of laboratory animals, and reactivity to human target cells can be obtained by immunizing with human target cells or by immunizing HLA-transgenic animals with the antigen/epitope. For some therapeutic approaches T cells derived from the same species are desirable. While such a cell can be created by cloning, for example, a murine TCR into a human T cell as contemplated above, *in vitro* immunization of human cells offers a potentially faster option. Techniques for *in vitro* immunization, even using naive donors, are known in the field, for example, Stauss et al., *Proc. Natl. Acad. Sci. USA* 89:7871-7875, 1992; Salgaller et al. *Cancer Res.* 55:4972-4979, 1995; Tsai et 30

al., *J. Immunol.* 158:1796-1802, 1997; and Chung et al., *J. Immunother.* 22:279-287, 1999; which are hereby incorporated by reference in their entirety.

Any of these molecules can be conjugated to enzymes, radiochemicals, fluorescent tags, and toxins, so as to be used in the diagnosis (imaging or other detection), monitoring, and treatment of the pathogenic condition associated with the epitope. Thus a toxin conjugate can be administered to kill tumor cells, radiolabeling can facilitate imaging of epitope positive tumor, an enzyme conjugate can be used in an ELISA-like assay to diagnose cancer and confirm epitope expression in biopsied tissue. In a further embodiment, such T cells as set forth above, following expansion accomplished through stimulation with the epitope and/or cytokines, can be administered to a patient as an adoptive immunotherapy.

Reagents Comprising Epitopes

A further aspect of the invention provides isolated epitope-MHC complexes. In a particularly advantageous embodiment of this aspect of the invention, the complexes can be soluble, multimeric proteins such as those described in U. S. Patent No. 5,635,363 (tetramers) or U. S. Patent No. 6,015,884 (Ig-dimers), both of which are hereby incorporated by reference in their entirety. Such reagents are useful in detecting and monitoring specific T cell responses, and in purifying such T cells.

Isolated MHC molecules complexed with epitopic peptides can also be incorporated into planar lipid bilayers or liposomes. Such compositions can be used to stimulate T cells *in vitro* or, in the case of liposomes, *in vivo*. Co-stimulatory molecules (e.g. B7, CD40, LFA-3) can be incorporated into the same compositions or, especially for *in vitro* work, co-stimulation can be provided by anti-co-receptor antibodies (e.g. anti-CD28, anti-CD154, anti-CD2) or cytokines (e.g. IL-2, IL-12). Such stimulation of T cells can constitute vaccination, drive expansion of T cells *in vitro* for subsequent infusion in an immunotherapy, or constitute a step in an assay of T cell function.

The epitope, or more directly its complex with an MHC molecule, can be an important constituent of functional assays of antigen-specific T cells at either an activation or readout step or both. Of the many assays of T cell function current in the art (detailed procedures can be found in standard immunological references such as *Current Protocols in Immunology* 1999 John Wiley & Sons Inc., N.Y., which is hereby incorporated by reference in its entirety) two broad classes can be defined, those that measure the response of a pool of cells and those that measure the response of individual cells. Whereas the former conveys a global measure of the strength of a response, the latter allows determination of the relative frequency of responding cells. Examples of assays measuring global response are cytotoxicity assays, ELISA, and proliferation assays detecting cytokine secretion. Assays measuring the responses of individual cells (or small clones derived from them) include limiting dilution analysis (LDA), ELISPOT, flow cytometric detection of

unsecreted cytokine (described in U.S. Patent No. 5,445,939, entitled "METHOD FOR ASSESSMENT OF THE MONONUCLEAR LEUKOCYTE IMMUNE SYSTEM" and U.S. Patent Nos 5,656,446; and 5,843,689, both entitled "METHOD FOR THE ASSESSMENT OF THE MONONUCLEAR LEUKOCYTE IMMUNE SYSTEM," reagents for which are sold by Becton, Dickinson & Company under the tradename 'FASTIMMUNE', which patents are hereby incorporated by reference in their entirety) and detection of specific TCR with tetramers or Ig-dimers as stated and referenced above. The comparative virtues of these techniques have been reviewed in Yee, C. et al. *Current Opinion in Immunology*, 13:141-146, 2001, which is hereby incorporated by reference in its entirety. Additionally detection of a specific TCR rearrangement or expression can be accomplished through a variety of established nucleic acid based techniques, particularly in situ and single-cell PCR techniques, as will be apparent to one of skill in the art.

These functional assays are used to assess endogenous levels of immunity, response to an immunologic stimulus (e.g. a vaccine), and to monitor immune status through the course of a disease and treatment. Except when measuring endogenous levels of immunity, any of these assays presume a preliminary step of immunization, whether *in vivo* or *in vitro* depending on the nature of the issue being addressed. Such immunization can be carried out with the various embodiments of the invention described above or with other forms of immunogen (e.g., pAPC-tumor cell fusions) that can provoke similar immunity. With the exception of PCR and tetramer/Ig-dimer type analyses which can detect expression of the cognate TCR, these assays generally benefit from a step of *in vitro* antigenic stimulation which can advantageously use various embodiments of the invention as described above in order to detect the particular functional activity (highly cytolytic responses can sometimes be detected directly). Finally, detection of cytolytic activity requires epitope-displaying target cells, which can be generated using various embodiments of the invention. The particular embodiment chosen for any particular step depends on the question to be addressed, ease of use, cost, and the like, but the advantages of one embodiment over another for any particular set of circumstances will be apparent to one of skill in the art.

Tumor Associated Antigens

Epitopes of the present invention are derived from the TuAAs tyrosinase (SEQ ID NO. 2), SSX-2, (SEQ ID NO. 3), PSMA (prostate-specific membrane antigen) (SEQ ID NO. 4), GP100, (SEQ ID NO. 70), MAGE-1, (SEQ ID NO. 71), MAGE-2, (SEQ ID NO. 72), MAGE-3, (SEQ ID NO. 73), NY-ESO-1, (SEQ ID NO. 74), PRAME, (SEQ ID NO. 77), PSA, (SEQ ID NO. 78), and PSCA, (SEQ ID NO. 79). The natural coding sequences for these eleven proteins, or any segments within them, can be determined from their cDNA or complete coding (cds) sequences, SEQ ID NOS. 5-7, and 80-87, respectively.

Tyrosinase is a melanin biosynthetic enzyme that is considered one of the most specific markers of melanocytic differentiation. Tyrosinase is expressed in few cell types, primarily in

melanocytes, and high levels are often found in melanomas. The usefulness of tyrosinase as a TuAA is taught in U.S. Patent 5,747,271 entitled "METHOD FOR IDENTIFYING INDIVIDUALS SUFFERING FROM A CELLULAR ABNORMALITY SOME OF WHOSE ABNORMAL CELLS PRESENT COMPLEXES OF HLA-A2/TYROSINASE DERIVED PEPTIDES, AND METHODS FOR TREATING SAID INDIVIDUALS" which is hereby incorporated by reference in its entirety.

GP100, also known as PMel17, also is a melanin biosynthetic protein expressed at high levels in melanomas. GP100 as a TuAA is disclosed in U.S. Patent 5,844,075 entitled "MELANOMA ANTIGENS AND THEIR USE IN DIAGNOSTIC AND THERAPEUTIC METHODS," which is hereby incorporated by reference in its entirety.

SSX-2, also known as Hom-Mel-40, is a member of a family of highly conserved cancer-testis antigens (Gure, A.O. et al. *Int. J. Cancer* 72:965-971, 1997, which is hereby incorporated by reference in its entirety). Its identification as a TuAA is taught in U.S. Patent 6,025,191 entitled "ISOLATED NUCLEIC ACID MOLECULES WHICH ENCODE A MELANOMA SPECIFIC ANTIGEN AND USES THEREOF," which is hereby incorporated by reference in its entirety. Cancer-testis antigens are found in a variety of tumors, but are generally absent from normal adult tissues except testis. Expression of different members of the SSX family have been found variously in tumor cell lines. Due to the high degree of sequence identity among SSX family members, similar epitopes from more than one member of the family will be generated and able to bind to an MHC molecule, so that some vaccines directed against one member of this family can cross-react and be effective against other members of this family (see example 3 below).

MAGE-1, MAGE-2, and MAGE-3 are members of another family of cancer-testis antigens originally discovered in melanoma (MAGE is a contraction of melanoma-associated antigen) but found in a variety of tumors. The identification of MAGE proteins as TuAAs is taught in U.S. Patent 5,342,774 entitled NUCLEOTIDE SEQUENCE ENCODING THE TUMOR REJECTION ANTIGEN PRECURSOR, MAGE-1, which is hereby incorporated by reference in its entirety, and in numerous subsequent patents. Currently there are 17 entries for (human) MAGE in the SWISS Protein database. There is extensive similarity among these proteins so in many cases, an epitope from one can induce a cross-reactive response to other members of the family. A few of these have not been observed in tumors, most notably MAGE-H1 and MAGE-D1, which are expressed in testes and brain, and bone marrow stromal cells, respectively. The possibility of cross-reactivity on normal tissue is ameliorated by the fact that they are among the least similar to the other MAGE proteins.

NY-ESO-1, is a cancer-testis antigen found in a wide variety of tumors, also known as CTAG-1 (Cancer-Testis Antigen-1) and CAG-3 (Cancer Antigen-3). NY-ESO-1 as a TuAA is disclosed in U.S. Patent 5,804,381 entitled ISOLATED NUCLEIC ACID MOLECULE

ENCODING AN ESOPHAGEAL CANCER ASSOCIATED ANTIGEN, THE ANTIGEN ITSELF,
AND USES THEREOF which is hereby incorporated by reference in its entirety. A paralogous
locus encoding antigens with extensive sequence identity, LAGE-1a/s (SEQ ID NO. 75) and
LAGE-1b/L (SEQ ID NO. 76), have been disclosed in publicly available assemblies of the human
genome, and have been concluded to arise through alternate splicing. Additionally, CT-2 (or
CTAG-2, Cancer-Testis Antigen-2) appears to be either an allele, a mutant, or a sequencing
discrepancy of LAGE-1b/L. Due to the extensive sequence identity, many epitopes from NY-ESO-
1 can also induce immunity to tumors expressing these other antigens. See figure 1. The proteins
are virtually identical through amino acid 70. From 71-134 the longest run of identities between
NY-ESO-1 and LAGE is 6 residues, but potentially cross-reactive sequences are present. And
from 135-180 NY-ESO and LAGE-1a/s are identical except for a single residue, but LAGE-1b/L is
unrelated due to the alternate splice. The CAMEL and LAGE-2 antigens appear to derive from the
LAGE-1 mRNA, but from alternate reading frames, thus giving rise to unrelated protein sequences.
More recently, GenBank Accession AF277315.5, Homo sapiens chromosome X clone RP5-
865E18, RP5-1087L19, complete sequence, reports three independent loci in this region which are
labeled as LAGE1 (corresponding to CTAG-2 in the genome assemblies), plus LAGE2-A and
LAGE2-B (both corresponding to CTAG-1 in the genome assemblies).

PSMA (prostate-specific membranes antigen), a TuAA described in U.S. Patent 5,538,866
entitled "PROSTATE-SPECIFIC MEMBRANES ANTIGEN" which is hereby incorporated by
reference in its entirety, is expressed by normal prostate epithelium and, at a higher level, in
prostatic cancer. It has also been found in the neovasculature of non-prostatic tumors. PSMA can
thus form the basis for vaccines directed to both prostate cancer and to the neovasculature of other
tumors. This later concept is more fully described in a provisional U.S. Patent application No.
60/274,063 entitled ANTI-NEOVASCULAR VACCINES FOR CANCER, filed March 7, 2001,
and U.S. Application No. __/____, attorney docket number CTLMM.015A, filed on March 7,
2002, entitled "ANTI-NEOVASCULAR PREPARATIONS FOR CANCER," both of which are
hereby incorporated by reference in their entirety. Alternate splicing of the PSMA mRNA also
leads to a protein with an apparent start at Met₅₈, thereby deleting the putative membrane anchor
region of PSMA as described in U.S. Patent 5,935,818 entitled "ISOLATED NUCLEIC ACID
MOLECULE ENCODING ALTERNATIVELY SPLICED PROSTATE-SPECIFIC
MEMBRANES ANTIGEN AND USES THEREOF" which is hereby incorporated by reference in
its entirety. A protein termed PSMA-like protein, Genbank accession number AF261715, is nearly
identical to amino acids 309-750 of PSMA and has a different expression profile. Thus the most
preferred epitopes are those with an N-terminus located from amino acid 58 to 308.

PRAME, also known as MAPE, DAGE, and OIP4, was originally observed as a melanoma
antigen. Subsequently, it has been recognized as a CT antigen, but unlike many CT antigens (e.g.,

MAGE, GAGE, and BAGE) it is expressed in acute myeloid leukemias. PRAME is a member of the MAPE family which consists largely of hypothetical proteins with which it shares limited sequence similarity. The usefulness of PRAME as a TuAA is taught in U.S. Patent 5,830,753 entitled "ISOLATED NUCLEIC ACID MOLECULES CODING FOR TUMOR REJECTION
5 ANTIGEN PRECURSOR DAGE AND USES THEREOF" which is hereby incorporated by reference in its entirety.

PSA, prostate specific antigen, is a peptidase of the kallikrein family and a differentiation antigen of the prostate. Expression in breast tissue has also been reported. Alternate names include gamma-seminoprotein, kallikrein 3, seminogelase, seminin, and P-30 antigen. PSA has a high
10 degree of sequence identity with the various alternate splicing products prostatic/glandular kallikrein-1 and -2, as well as kalikrein 4, which is also expressed in prostate and breast tissue. Other kallikreins generally share less sequence identity and have different expression profiles. Nonetheless, cross-reactivity that might be provoked by any particular epitope, along with the likelihood that that epitope would be liberated by processing in non-target tissues (most generally
15 by the housekeeping proteasome), should be considered in designing a vaccine.

PSCA, prostate stem cell antigen, and also known as SCAH-2, is a differentiation antigen preferentially expressed in prostate epithelial cells, and overexpressed in prostate cancers. Lower level expression is seen in some normal tissues including neuroendocrine cells of the digestive tract and collecting ducts of the kidney. PSCA is described in U.S. Patent 5,856,136 entitled "HUMAN
20 STEM CELL ANTIGENS" which is hereby incorporated by reference in its entirety.

Synaptonemal complex protein 1 (SCP-1), also known as HOM-TES-14, is a meiosis-associated protein and also a cancer-testis antigen (Tureci, O., et al. *Proc. Natl. Acad. Sci. USA* 95:5211-5216, 1998). As a cancer antigen its expression is not cell-cycle regulated and it is found frequently in gliomas, breast, renal cell, and ovarian carcinomas. It has some similarity to myosins,
25 but with few enough identities that cross-reactive epitopes are not an immediate prospect.

The ED-B domain of fibronectin is also a potential target. Fibronectin is subject to developmentally regulated alternative splicing, with the ED-B domain being encoded by a single exon that is used primarily in oncofetal tissues (Matsuura, H. and S. Hakomori *Proc. Natl. Acad. Sci. USA* 82:6517-6521, 1985; Carnemolla, B. et al. *J. Cell Biol.* 108:1139-1148, 1989; Lordon-
30 Rosa, B. et al. *Cancer Res.* 50:1608-1612, 1990; Nicolo, G. et al. *Cell Differ. Dev.* 32:401-408, 1990; Borsi, L. et al. *Exp. Cell Res.* 199:98-105, 1992; Oyama, F. et al. *Cancer Res.* 53:2005-2011, 1993; Mandel, U. et al. *APMIS* 102:695-702, 1994; Farnoud, M.R. et al. *Int. J. Cancer* 61:27-34, 1995; Pujuguet, P. et al. *Am. J. Pathol.* 148:579-592, 1996; Gabler, U. et al. *Heart* 75:358-362, 1996; Chevalier, X. *Br. J. Rheumatol.* 35:407-415, 1996; Midulla, M. *Cancer Res.* 60:164-169,
35 2000).

The ED-B domain is also expressed in fibronectin of the neovasculature (Kaczmarek, J. et al. *Int. J. Cancer* 59:11-16, 1994; Castellani, P. et al. *Int. J. Cancer* 59:612-618, 1994; Neri, D. et al. *Nat. Biotech.* 15:1271-1275, 1997; Karelina, T.V. and A.Z. Eisen *Cancer Detect. Prev.* 22:438-444, 1998; Tarli, L. et al. *Blood* 94:192-198, 1999; Castellani, P. et al. *Acta Neurochir. (Wien)* 142:277-282, 2000). As an oncofetal domain, the ED-B domain is commonly found in the fibronectin expressed by neoplastic cells in addition to being expressed by the neovasculature. Thus, CTL-inducing vaccines targeting the ED-B domain can exhibit two mechanisms of action: direct lysis of tumor cells, and disruption of the tumor's blood supply through destruction of the tumor-associated neovasculature. As CTL activity can decay rapidly after withdrawal of vaccine, interference with normal angiogenesis can be minimal. The design and testing of vaccines targeted to neovasculature is described in Provisional U.S. Patent Application No. 60/274,063 entitled "ANTI-NEOVASCULATURE VACCINES FOR CANCER" and in U.S. Patent Application No. ____/____, attorney docket number CTLIMM.015A, entitled "ANTI-NEOVASCULATURE PREPARATIONS FOR CANCER, filed on date even with this application (March 7, 2002). A tumor cell line is disclosed in Provisional U.S. Application No. ____/____, filed on March 7, 2002, attorney docket number CTLIMM.028PR, entitled "HLA-TRANSGENIC MURINE TUMOR CELL LINE," which is hereby incorporated by reference in its entirety.

Carcinoembryonic antigen (CEA) is a paradigmatic oncofetal protein first described in 1965 (Gold and Freedman, J. Exp. Med. 121: 439-462, 1965. Fuller references can be found in the Online Medelian Inheritance in Man; record *114890). It has officially been renamed carcinoembryonic antigen-related cell adhesion molecule 5 (CEACAM5). Its expression is most strongly associated with adenocarcinomas of the epithelial lining of the digestive tract and in fetal colon. CEA is a member of the immunoglobulin supergene family and the defining member of the CEA subfamily.

HER2/NEU is an oncogene related to the epidermal growth factor receptor (van de Vijver, et al., *New Eng. J. Med.* 319:1239-1245, 1988), and apparently identical to the c-ERBB2 oncogene (Di Fiore, et al., *Science* 237: 178-182, 1987). The over-expression of ERBB2 has been implicated in the neoplastic transformation of prostate cancer. As HER2 it is amplified and over-expressed in 25-30% of breast cancers among other tumors where expression level is correlated with the aggressiveness of the tumor (Slamon, et al., *New Eng. J. Med.* 344:783-792, 2001). A more detailed description is available in the Online Medelian Inheritance in Man; record *164870.

All references mentioned herein are hereby incorporated by reference in their entirety. Further, incorporated by reference in its entirety is U.S. Patent Application No. 10/005,905 (attorney docket number CTLIMM.021CP1) entitled "EPITOPE SYNCHRONIZATION IN ANTIGEN PRESENTING CELLS," filed on November 7, 2001 and a continuation thereof, U.S. Application No. ____/____, filed on December 7, 2000, attorney docket number

CTLIMM.21CP1C, also entitled "EPI TOPE SYNCHRONIZATION IN ANTIGEN PRESENTING CELLS."

Useful epitopes were identified and tested as described in the following examples. However, these examples are intended for illustration purposes only, and should not be construed as limiting the scope of the invention in any way.

EXAMPLES

Sequences of Specific Preferred Epitopes

Example 1

Manufacture of tyrosinase epitopes.

A. Synthetic production of epitopes

Peptides having an amino acid sequence of any of SEQ ID NO: 1, 8, 9, 11-23, 2-29, 32-44, 47-54, 56-63, 66-68 88-253, or 256-588 are synthesized using either FMOC or tBOC solid phase synthesis methodologies. After synthesis, the peptides are cleaved from their supports with either trifluoroacetic acid or hydrogen fluoride, respectively, in the presence of appropriate protective scavengers. After removing the acid by evaporation, the peptides are extracted with ether to remove the scavengers and the crude, precipitated peptide is then lyophilized. Purity of the crude peptides is determined by HPLC, sequence analysis, amino acid analysis, counterion content analysis and other suitable means. If the crude peptides are pure enough (greater than or equal to about 90% pure), they can be used as is. If purification is required to meet drug substance specifications, the peptides are purified using one or a combination of the following: re-precipitation; reverse-phase, ion exchange, size exclusion or hydrophobic interaction chromatography; or counter-current distribution.

Drug product formulation

GMP-grade peptides are formulated in a parenterally acceptable aqueous, organic, or aqueous-organic buffer or solvent system in which they remain both physically and chemically stable and biologically potent. Generally, buffers or combinations of buffers or combinations of buffers and organic solvents are appropriate. The pH range is typically between 6 and 9. Organic modifiers or other excipients can be added to help solubilize and stabilize the peptides. These include detergents, lipids, co-solvents, antioxidants, chelators and reducing agents. In the case of a lyophilized product, sucrose or mannitol or other lyophilization aids can be added. Peptide solutions are sterilized by membrane filtration into their final container-closure system and either lyophilized for dissolution in the clinic, or stored until use.

B. Construction of expression vectors for use as nucleic acid vaccines

The construction of three generic epitope expression vectors is presented below. The particular advantages of these designs are set forth in U.S. Patent Application No. 09/561,572

entitled "EXPRESSION VECTORS ENCODING EPITOPES OF TARGET-ASSOCIATED ANTIGENS," which has been incorporated by reference in its entirety above.

A suitable *E. coli* strain was then transfected with the plasmid and plated out onto a selective medium. Several colonies were grown up in suspension culture and positive clones were identified by restriction mapping. The positive clone was then grown up and aliquotted into storage vials and stored at -70°C.

A mini-prep (QIAprep Spin Mini-prep: Qiagen, Valencia, CA) of the plasmid was then made from a sample of these cells and automated fluorescent dideoxy sequence analysis was used to confirm that the construct had the desired sequence.

B.1 Construction of pVAX-EP1-IRES-EP2

Overview:

The starting plasmid for this construct is pVAX1 purchased from Invitrogen (Carlsbad, CA). Epitopes EP1 and EP2 were synthesized by GIBCO BRL (Rockville, MD). The IRES was excised from pIRES purchased from Clontech (Palo Alto, CA).

Procedure:

1 pIRES was digested with EcoRI and NotI. The digested fragments were separated by agarose gel electrophoresis, and the IRES fragment was purified from the excised band.

2 pVAX1 was digested with EcoRI and NotI, and the pVAX1 fragment was gel-purified.

3 The purified pVAX1 and IRES fragments were then ligated together.

4 Competent *E. coli* of strain DH5 α were transformed with the ligation mixture.

5 Minipreps were made from 4 of the resultant colonies.

6 Restriction enzyme digestion analysis was performed on the miniprep DNA. One recombinant colony having the IRES insert was used for further insertion of EP1 and EP2. This intermediate construct was called pVAX-IRES.

7 Oligonucleotides encoding EP1 and EP2 were synthesized.

8 EP1 was subcloned into pVAX-IRES between AflII and EcoRI sites, to make pVAX-EP1-IRES;

9 EP2 was subcloned into pVAX-EP1-IRES between SalI and NotI sites, to make the final construct pVAX-EP1-IRES-EP2.

10 The sequence of the EP1-IRES-EP2 insert was confirmed by DNA sequencing.

B.2. Construction of pVAX-EP1-IRES-EP2-ISS-NIS

Overview:

The starting plasmid for this construct was pVAX-EP1-IRES-EP2 (Example 1). The ISS (immunostimulatory sequence) introduced into this construct is AACGTT, and the NIS (standing for nuclear import sequence) used is the SV40 72bp repeat sequence. ISS-NIS was synthesized by GIBCO BRL. See Figure 2.

Procedure:

- 1 pVAX-EP1-IRES-EP2 was digested with NruI; the linearized plasmid was gel-purified.
- 2 ISS-NIS oligonucleotide was synthesized.
- 3 The purified linearized pVAX-EP1-IRES-EP2 and synthesized ISS-NIS were ligated
5 together.
- 4 Competent E. coli of strain DH5 α were transformed with the ligation product.
- 5 Minipreps were made from resultant colonies.
- 6 Restriction enzyme digestions of the minipreps were carried out.
- 7 The plasmid with the insert was sequenced.

10 **B3. Construction of pVAX-EP2-UB-EP1**

Overview:

The starting plasmid for this construct was pVAX1 (Invitrogen). EP2 and EP1 were synthesized by GIBCO BRL. Wild type Ubiquitin cDNA encoding the 76 amino acids in the construct was cloned from yeast.

15 **Procedure:**

- 1 RT-PCR was performed using yeast mRNA. Primers were designed to amplify the complete coding sequence of yeast Ubiquitin.
- 2 The RT-PCR products were analyzed using agarose gel electrophoresis. A band with the predicted size was gel-purified.
- 20 3 The purified DNA band was subcloned into pZERO1 at EcoRV site. The resulting clone was named pZERO-UB.
- 4 Several clones of pZERO-UB were sequenced to confirm the Ubiquitin sequence before further manipulations.
- 5 EP1 and EP2 were synthesized.
- 25 6 EP2, Ubiquitin and EP1 were ligated and the insert cloned into pVAX1 between BamHI and EcoRI, putting it under control of the CMV promoter.
- 7 The sequence of the insert EP2-UB-EP1 was confirmed by DNA sequencing.

Example 2**Identification of useful epitope variants.**

- 30 The 10-mer FLPWHRLFLL (SEQ ID NO. 1) is identified as a useful epitope. Based on this sequence, numerous variants are made. Variants exhibiting activity in HLA binding assays (see Example 3, section 6) are identified as useful, and are subsequently incorporated into vaccines.

- The HLA-A2 binding of length variants of FLPWHRLFLL have been evaluated. Proteasomal digestion analysis indicates that the C-terminus of the 9-mer FLPWHRLFL (SEQ ID
35 NO. 8) is also produced. Additionally the 9-mer LPWHRLFLL (SEQ ID NO. 9) can result from N-terminal trimming of the 10-mer. Both are predicted to bind to the HLA-A*0201 molecule,

however of these two 9-mers, FLPWHRLFL displayed more significant binding and is preferred (see Figs. 3A and B).

Sequence variants of FLPWHRLFL are constructed as follow. Consistent with the binding coefficient table (see Table 3) from the NIH/BIMAS MHC binding prediction program (see reference in example 3 below), binding can be improved by changing the L at position 9, an anchor position, to V. Binding can also be altered, though generally to a lesser extent, by changes at non-anchor positions. Referring generally to Table 3, binding can be increased by employing residues with relatively larger coefficients. Changes in sequence can also alter immunogenicity independently of their effect on binding to MHC. Thus binding and/or immunogenicity can be improved as follows:

By substituting F, L, M, W, or Y for P at position 3; these are all bulkier residues that can also improve immunogenicity independent of the effect on binding. The amine and hydroxyl-bearing residues, Q and N; and S and T; respectively, can also provoke a stronger, cross-reactive response.

By substituting D or E for W at position 4 to improve binding; this addition of a negative charge can also make the epitope more immunogenic, while in some cases reducing cross-reactivity with the natural epitope. Alternatively the conservative substitutions of F or Y can provoke a cross-reactive response.

By substituting F for H at position 5 to improve binding. H can be viewed as partially charged, thus in some cases the loss of charge can hinder cross-reactivity. Substitution of the fully charged residues R or K at this position can enhance immunogenicity without disrupting charge-dependent cross-reactivity.

By substituting I, L, M, V, F, W, or Y for R at position 6. The same caveats and alternatives apply here as at position 5.

By substituting W or F for L at position 7 to improve binding. Substitution of V, I, S, T, Q, or N at this position are not generally predicted to reduce binding affinity by this model (the NIH algorithm), yet can be advantageous as discussed above.

Y and W, which are equally preferred as the Fs at positions 1 and 8, can provoke a useful cross-reactivity. Finally, while substitutions in the direction of bulkiness are generally favored to improve immunogenicity, the substitution of smaller residues such as A, S, and C, at positions 3-7 can be useful according to the theory that contrast in size, rather than bulkiness per se, is an important factor in immunogenicity. The reactivity of the thiol group in C can introduce other properties as discussed in Chen, J.-L., et al. *J. Immunol.* 165:948-955, 2000.

Table 3. 9-mer Coefficient Table for HLA-A*0201*

HLA Coefficient table for file "A_0201_standard"									
Amino Acid Type	1 st	2 nd	3 rd	4 th	5 th	6 th	7 th	8 th	9 th
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.00
C	1.000	0.470	1.000	1.000	1.000	1.000	1.000	1.000	1.00
D	0.075	0.100	0.400	4.100	1.000	1.000	0.490	1.000	0.00
E	0.075	1.400	0.064	4.100	1.000	1.000	0.490	1.000	0.00
F	4.600	0.050	3.700	1.000	3.800	1.900	5.800	5.500	0.01
G	1.000	0.470	1.000	1.000	1.000	1.000	0.130	1.000	0.01
H	0.034	0.050	1.000	1.000	1.000	1.000	1.000	1.000	0.01
I	1.700	9.900	1.000	1.000	1.000	2.300	1.000	0.410	2.10
K	3.500	0.100	0.035	1.000	1.000	1.000	1.000	1.000	0.00
L	1.700	72.000	3.700	1.000	1.000	2.300	1.000	1.000	4.30
M	1.700	52.000	3.700	1.000	1.000	2.300	1.000	1.000	1.00
N	1.000	0.470	1.000	1.000	1.000	1.000	1.000	1.000	0.01
P	0.022	0.470	1.000	1.000	1.000	1.000	1.000	1.000	0.00
Q	1.000	7.300	1.000	1.000	1.000	1.000	1.000	1.000	0.00
R	1.000	0.010	0.076	1.000	1.000	1.000	0.200	1.000	0.00
S	1.000	0.470	1.000	1.000	1.000	1.000	1.000	1.000	0.01
T	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.50
V	1.700	6.300	1.000	1.000	1.000	2.300	1.000	0.410	14.00
W	4.600	0.010	8.300	1.000	1.000	1.700	7.500	5.500	0.01
Y	4.600	0.010	3.200	1.000	1.000	1.500	1.000	5.500	0.01

*This table and other comparable data that are publicly available are useful in designing epitope variants and in determining whether a particular variant is substantially similar, or is functionally similar.

Example 3

Cluster Analysis (SSX-2₃₁₋₆₈).

1. Epitope cluster region prediction:

The computer algorithms: SYFPEITHI (internet [http:// syfpeithi.bmi-heidelberg.com/Scripts/MHCServer.dll/EpPredict.htm](http://syfpeithi.bmi-heidelberg.com/Scripts/MHCServer.dll/EpPredict.htm)), based on the book "MHC Ligands and Peptide Motifs" by H.G.Rammensee, J.Bachmann and S.Stevanovic; and HLA Peptide Binding Predictions (NIH) (internet [http:// access at bimas.dcrt.nih.gov/molbio/hla_bin](http://bimas.dcrt.nih.gov/molbio/hla_bin)), described in Parker, K. C., et al., *J. Immunol.* 152:163, 1994; were used to analyze the protein sequence of SSX-2 (GI:10337583). Epitope clusters (regions with higher than average density of peptide fragments with high predicted MHC affinity) were defined as described fully in U.S. Patent Application No. 09/561,571 entitled "EPITOPE CLUSTERS," filed on April 28, 2000. Using a epitope density ratio cutoff of 2, five and two clusters were defined using the SYFPEITHI and NIH algorithms, respectively, and peptides score cutoffs of 16 (SYFPEITHI) and 5 (NIH). The highest scoring peptide with the NIH algorithm, SSX-2₄₁₋₄₉, with an estimated half-time of dissociation of

>1000 min., does not overlap any other predicted epitope but does cluster with SSX-2₅₇₋₆₅ in the NIH analysis.

2. Peptide synthesis and characterization:

SSX-2₃₁₋₆₈, YFSKEEWEKMKASEKIFYVYMKRKYEAMTKLGFKATLP (SEQ ID NO. 10) was synthesized by MPS (Multiple Peptide Systems, San Diego, CA 92121) using standard solid phase chemistry. According to the provided 'Certificate of Analysis', the purity of this peptide was 95%.

3. Proteasome digestion:

Proteasome was isolated from human red blood cells using the proteasome isolation protocol described in U.S. Patent Application No. 09/561,074 entitled "METHOD OF EPITOPE DISCOVERY," filed on April 28, 2000. SDS-PAGE, western-blotting, and ELISA were used as quality control assays. The final concentration of proteasome was 4 mg/ml, which was determined by non-interfering protein assay (Geno Technologies Inc.). Proteasomes were stored at -70°C in 25 µl aliquots.

SSX-2₃₁₋₆₈ was dissolved in Milli-Q water, and a 2 mM stock solution prepared and 20µL aliquots stored at -20°C.

1 tube of proteasome (25 µL) was removed from storage at -70°C and thawed on ice. It was then mixed thoroughly with 12.5µL of 2mM peptide by repipetting (samples were kept on ice). A 5µL sample was immediately removed after mixing and transferred to a tube containing 1.25µL 10%TFA (final concentration of TFA was 2%); the T=0 min sample. The proteasome digestion reaction was then started and carried out at 37°C in a programmable thermal controller. Additional 5µL samples were taken out at 15, 30, 60, 120, 180 and 240 min respectively, the reaction was stopped by adding the sample to 1.25µL 10% TFA as before. Samples were kept on ice or frozen until being analyzed by MALDI-MS. All samples were saved and stored at -20°C for HPLC analysis and N-terminal sequencing. Peptide alone (without proteasome) was used as a blank control: 2 µL peptide + 4µL Tris buffer (20 mM, pH 7.6) + 1.5µL TFA.

4. MALDI-TOF MS measurements:

For each time point 0.3 µL of matrix solution (10mg/ml α-cyano-4-hydroxycinnamic acid in AcCN/H₂O (70:30)) was first applied on a sample slide, and then an equal volume of digested sample was mixed gently with matrix solution on the slide. The slide was allowed to dry at ambient air for 3-5 min. before acquiring the mass spectra. MS was performed on a Lasermat 2000 MALDI-TOF mass spectrometer that was calibrated with peptide/protein standards. To improve the accuracy of measurement, the molecular ion weight (MH⁺) of the peptide substrate was used as an internal calibration standard. The mass spectrum of the T=120 min. digested sample is shown in figure 4.

5. MS data analysis and epitope identification:

To assign the measured mass peaks, the computer program MS-Product, a tool from the UCSF Mass Spectrometry Facility (<http://prospector.ucsf.edu/ucsfhtml3.4/msprod.htm>), was used to generate all possible fragments (N- and C-terminal ions, and internal fragments) and their corresponding molecular weights. Due to the sensitivity of the mass spectrometer, average molecular weight was used. The mass peaks observed over the course of the digestion were identified as summarized in Table 4.

Fragments co-C-terminal with 8-10 amino acid long sequences predicted to bind HLA by the SYFPEITHI or NIH algorithms were chosen for further study. The digestion and prediction steps of the procedure can be usefully practiced in any order. Although the substrate peptide used in proteasomal digest described here was specifically designed to include predicted HLA-A2.1 binding sequences, the actual products of digestion can be checked after the fact for actual or predicted binding to other MHC molecules. Selected results are shown in Table 5.

Table 4. SSX-2₃₁₋₆₈ Mass Peak Identification.

MS PEAK (measured)	PEPTIDE	SEQUENCE	CALCULATED MASS (MH ⁺)
988.23	31-37	YFSKBEW	989.08
1377.68±2.3 8	31-40	YFSKEEWEKM	1377.68
1662.45±1.3 0	31-43	YFSKEEWEKMKAS	1663.90
2181.72±0.8 5	31-47	YFSKEEWEKMKASEKIF	2181.52
2346.6	31-48	YFSKEEWEKMKASEKIFY	2344.71
1472.16±1.5 4	38-49	EKMKASEKIFYV	1473.77
2445.78±1.1 8	31-49*	YFSKEEWEKMKASEKIFYV	2443.84
2607.	31-50	YFSKEEWEKMKASEKIFYVY	2607.02
1563.3	50-61	YMKRKYEAMTKL	1562.93
3989.9	31-61	YFSKEEWEKMKASEKIFYVYMKRKYEAMTKL	3987.77
1603.74±1.5 3	51-63	MKRKYEAMTKLGF	1603.98
1766.45±1.5	50-63	YMKRKYEAMTKLGF	1767.16
1866.32±1.2 2	49-63	VYMKRKYEAMTKLGF	1866.29
4192.6	31-63	YFSKEEWEKMKASEKIFYVYMKRKYEAMTKLG F	4192.00
4392.1	31-65**	YFSKEEWEKMKASEKIFYVYMKRKYEAMTKLG FKA	4391.25

Boldface sequence correspond to peptides predicted to bind to MHC.

* On the basis of mass alone this peak could also have been assigned to the peptide 32-50, however proteasomal removal of just the N-terminal amino acid is unlikely. N-terminal sequencing (below) verifies the assignment to 31-49.

** On the basis of mass this fragment might also represent 33-68. N-terminal sequencing below is consistent with the assignment to 31-65.

5

Table 5. Predicted HLA binding by proteasomally generated fragments

<u>SEQ ID NO.</u>	<u>PEPTIDE</u>	<u>HLA</u>	<u>SYFPEITHI</u>	<u>NIH</u>
11	FSKEEWEKM	B*3501	NP†	90
12	KMKASEKIF	B*08	17	<5
13 & (14)	(K) MKASEKIFY	A1	19 (19)	<5
15 & (16)	(M) KASEKIFYV	A*0201	22 (16)	1017
		B*08	17	<5
		B*5101	22 (13)	60
		B*5102	NP	133
		B*5103	NP	121
17 & (18)	(K) ASEKIFYVY	A1	34 (19)	14
19 & (20)	(K) RKYEAMTKL	A*0201	15	<5
		A26	15	NP
		B14	NP	45 (60)
		B*2705	21	15
		B*2709	16	NP
		B*5101	15	<5
21	KYEAMTKLGF	A1	16	<5
		A24	NP	300
		B*4403	NP	80
22	YEAMTKLGF	B*08	22	<5
23	EAMTKLGF			

†No prediction

5

As seen in Table 5, N-terminal addition of authentic sequence to epitopes can generate epitopes for the same or different MHC restriction elements. Note in particular the pairing of (K)RKYEAMTKL (SEQ ID NOS 19 and (20)) with HLA-B14, where the 10-mer has a longer predicted halftime of dissociation than the co-C-terminal 9-mer. Also note the case of the 10-mer KYEAMTKLGF (SEQ ID NO. 21) which can be used as a vaccine useful with several MHC types by relying on N-terminal trimming to create the epitopes for HLA-B*4403 and -B*08.

10

6. HLA-A0201 binding assay:

Binding of the candidate epitope KASEKIFYV, SSX-2₄₁₋₄₉, (SEQ ID NO. 15) to HLA-A2.1 was assayed using a modification of the method of Stauss et al., (Proc Natl Acad Sci USA 89(17):7871-5 (1992)). Specifically, T2 cells, which express empty or unstable MHC molecules on their surface, were washed twice with Iscove's modified Dulbecco's medium (IMDM) and cultured overnight in serum-free AIM-V medium (Life Technologies, Inc., Rockville, MD) supplemented with human β 2-microglobulin at 3 μ g/ml (Sigma, St. Louis, MO) and added peptide,

15

at 800, 400, 200, 100, 50, 25, 12.5, and 6.25 $\mu\text{g/ml}$ in a 96-well flat-bottom plate at 3×10^5 cells/200 μl /well. Peptide was mixed with the cells by repipeting before distributing to the plate (alternatively peptide can be added to individual wells), and the plate was rocked gently for 2 minutes. Incubation was in a 5% CO_2 incubator at 37°C . The next day the unbound peptide was removed by washing twice with serum free RPMI medium and a saturating amount of anti-class I HLA monoclonal antibody, fluorescein isothiocyanate (FITC)-conjugated anti-HLA A2, A28 (One Lambda, Canoga Park, CA) was added. After incubation for 30 minutes at 4°C , cells were washed 3 times with PBS supplemented with 0.5% BSA, 0.05%(w/v) sodium azide, pH 7.4-7.6 (staining buffer). (Alternatively W6/32 (Sigma) can be used as the anti-class I HLA monoclonal antibody the cells washed with staining buffer and then incubated with fluorescein isothiocyanate (FITC)-conjugated goat F(ab') antimouse-IgG (Sigma) for 30 min at 4°C and washed 3 times as before.) The cells were resuspended in 0.5 ml staining buffer. The analysis of surface HLA-A2.1 molecules stabilized by peptide binding was performed by flow cytometry using a FACScan (Becton Dickinson, San Jose, CA). If flow cytometry is not to be performed immediately the cells can be fixed by adding a quarter volume of 2% paraformaldehyde and storing in the dark at 4°C .

The results of the experiment are shown in Figure 5. SSX-2₄₁₋₄₉ (SEQ ID NO. 15) was found to bind HLA-A2.1 to a similar extent as the known A2.1 binder FLPSDYFPSV (HBV₁₈₋₂₇; SEQ ID NO: 24) used as a positive control. An HLA-B44 binding peptide, AEMGKYSFY (SEQ ID NO: 25), was used as a negative control. The fluorescence obtained from the negative control was similar to the signal obtained when no peptide was used in the assay. Positive and negative control peptides were chosen from Table 18.3.1 in *Current Protocols in Immunology* p. 18.3.2, John Wiley and Sons, New York, 1998.

7. Immunogenicity:

A. In vivo immunization of mice.

HHD1 transgenic A*0201 mice (Pascolo, S., et al. *J. Exp. Med.* 185:2043-2051, 1997) were anesthetized and injected subcutaneously at the base of the tail, avoiding lateral tail veins, using 100 μl containing 100 nmol of SSX-2₄₁₋₄₉ (SEQ ID NO. 15) and 20 μg of HTL epitope peptide in PBS emulsified with 50 μl of IFA (incomplete Freund's adjuvant).

B. Preparation of stimulating cells (LPS blasts).

Using spleens from 2 naive mice for each group of immunized mice, un-immunized mice were sacrificed and the carcasses were placed in alcohol. Using sterile instruments, the top dermal layer of skin on the mouse's left side (lower mid-section) was cut through, exposing the peritoneum. The peritoneum was saturated with alcohol, and the spleen was aseptically extracted. The spleen was placed in a petri dish with serum-free media. Splenocytes were isolated by using sterile plungers from 3 ml syringes to mash the spleens. Cells were collected in a 50 ml conical tubes in serum-free media, rinsing dish well. Cells were centrifuged (12000 rpm, 7 min) and

washed one time with RPMI. Fresh spleen cells were resuspended to a concentration of 1×10^6 cells per ml in RPMI-10%FCS (fetal calf serum). 25g/ml lipopolysaccharide and 7 μ g/ml Dextran Sulfate were added. Cell were incubated for 3 days in T-75 flasks at 37°C, with 5% CO₂. Splenic blasts were collected in 50 ml tubes pelleted (12000 rpm, 7 min) and resuspended to 3×10^7 /ml in RPMI. The blasts were pulsed with the priming peptide at 50 μ g/ml, RT 4hr. mitomycin C-treated at 25 μ g/ml, 37°C, 20 min and washed three times with DMEM.

C. In vitro stimulation.

3 days after LPS stimulation of the blast cells and the same day as peptide loading, the primed mice were sacrificed (at 14 days post immunization) to remove spleens as above. 3×10^6 splenocytes were co-cultured with 1×10^6 LPS blasts/well in 24-well plates at 37°C, with 5% CO₂ in DMEM media supplemented with 10% FCS, 5×10^{-5} M β -mercaptoethanol, 100 μ g/ml streptomycin and 100 IU/ml penicillin. Cultures were fed 5% (vol/vol) ConA supernatant on day 3 and assayed for cytolytic activity on day 7 in a ⁵¹Cr-release assay.

D. Chromium-release assay measuring CTL activity.

To assess peptide specific lysis, 2×10^6 T2 cells were incubated with 100 μ Ci sodium chromate together with 50 μ g/ml peptide at 37 °C for 1 hour. During incubation they were gently shaken every 15 minutes. After labeling and loading, cells were washed three times with 10 ml of DMEM-10% FCS, wiping each tube with a fresh Kimwipe after pouring off the supernatant. Target cells were resuspended in DMEM-10% FBS 1×10^5 /ml. Effector cells were adjusted to 1×10^7 /ml in DMEM-10% FCS and 100 μ l serial 3-fold dilutions of effectors were prepared in U-bottom 96-well plates. 100 μ l of target cells were added per well. In order to determine spontaneous release and maximum release, six additional wells containing 100 μ l of target cells were prepared for each target. Spontaneous release was revealed by incubating the target cells with 100 μ l medium; maximum release was revealed by incubating the target cells with 100 μ l of 2% SDS. Plates were then centrifuged for 5 min at 600 rpm and incubated for 4 hours at 37°C in 5% CO₂ and 80% humidity. After the incubation, plates were then centrifuged for 5 min at 1200 rpm. Supernatants were harvested and counted using a gamma counter. Specific lysis was determined as follows: % specific release = [(experimental release - spontaneous release)/(maximum release - spontaneous release)] x 100.

Results of the chromium release assay demonstrating specific lysis of peptide pulsed target cells are shown in figure 6.

8. Cross-reactivity with other SSX proteins:

SSX-2₄₁₋₄₉ (SEQ ID NO. 15) shares a high degree of sequence identity with the same region of the other SSX proteins. The surrounding regions have also been generally well conserved. Thus the housekeeping proteasome can cleave following V₄₉ in all five sequences. Moreover, SSX₄₁₋₄₉ is

predicted to bind HLA-A*0201 (see Table 6). CTL generated by immunization with SSX-2₄₁₋₄₉ cross-react with tumor cells expressing other SSX proteins.

Table 6. SSX₄₁₋₄₉ – A*0201 Predicted Binding

SEQ ID NO.	Family Member	Sequence	SYFPEITHI Score	NIH Score
15	SSX-2	KASEKIFYV	22	1017
26	SSX-1	KYSEKISYV	18	1.7
27	SSX-3	KVSEKIVYV	24	1105
28	SSX-4	KSSEKIVYV	20	82
29	SSX-5	KASEKIYV	22	175

5 Example 4

Cluster Analysis (PSMA₁₆₃₋₁₉₂).

A peptide, AFSPQGMPEGDLVYVNYARTEDFFKLERDM, PSMA₁₆₃₋₁₉₂, (SEQ ID NO. 30), containing an A1 epitope cluster from prostate specific membrane antigen, PSMA₁₆₈₋₁₉₀ (SEQ ID NO. 31) was synthesized using standard solid-phase F-moc chemistry on a 433A ABI Peptide synthesizer. After side chain deprotection and cleavage from the resin, peptide first dissolved in formic acid and then diluted into 30% Acetic acid, was run on a reverse-phase preparative HPLC C4 column at following conditions: linear AB gradient (5% B/min) at a flow rate of 4 ml/min, where eluent A is 0.1% aqueous TFA and eluent B is 0.1% TFA in acetonitrile. A fraction at time 16.642 min containing the expected peptide, as judged by mass spectrometry, was pooled and lyophilized. The peptide was then subjected to proteasome digestion and mass spectrum analysis essentially as described above. Prominent peaks from the mass spectra are summarized in Table 7.

Table 7. PSMA₁₆₃₋₁₉₂ Mass Peak Identification.

PEPTIDE	SEQUENCE	CALCULATE D MASS (MH ⁺)
163-177	AFSPQGMPEGDLVYV	1610.0
178-189	NYARTEDFFKLE	1533.68
170-189	PEGDLVYVNYARTEDFFKLE	2406.66
178-191	NYARTEDFFKLERD	1804.95
170-191	PEGDLVYVNYARTEDFFKLERD	2677.93
178-192	NYARTEDFFKLERDM	1936.17
163-176	AFSPQGMPEGDLVY	1511.70
177-192	VNYARTEDFFKLERDM	2035.30
163-179	AFSPQGMPEGDLVYVNY	1888.12

180-192	ARTEDFFKLERDM	1658.89
163-183	AFSPQGMPEGDLVYVNYARTE	2345.61
184-192	DDFFKLERDM	1201.40
176-192	YVNYARTEDFFKLERDM	2198.48
167-185	QGMPEGDLVYVNYARTEDF	2205.41
178-186	NYARTEDFF	1163.22

Boldface sequences correspond to peptides predicted to bind to MHC, see Table 8.

N-terminal Pool Sequence Analysis

One aliquot at one hour of the proteasomal digestion (see Example 3 part 3 above) was subjected to N-terminal amino acid sequence analysis by an ABI 473A Protein Sequencer (Applied Biosystems, Foster City, CA). Determination of the sites and efficiencies of cleavage was based on consideration of the sequence cycle, the repetitive yield of the protein sequencer, and the relative yields of amino acids unique in the analyzed sequence. That is if the unique (in the analyzed sequence) residue X appears only in the nth cycle a cleavage site exists n-1 residues before it in the N-terminal direction. In addition to helping resolve any ambiguity in the assignment of mass to sequences, these data also provide a more reliable indication of the relative yield of the various fragments than does mass spectrometry.

For PSMA₁₆₃₋₁₉₂ (SEQ ID NO. 30) this pool sequencing supports a single major cleavage site after V₁₇₇ and several minor cleavage sites, particularly one after Y₁₇₉. Reviewing the results presented in figures 7A-C reveals the following:

- S at the 3rd cycle indicating presence of the N-terminus of the substrate.
 - Q at the 5th cycle indicating presence of the N-terminus of the substrate.
 - N at the 1st cycle indicating cleavage after V₁₇₇.
 - N at the 3rd cycle indicating cleavage after V₁₇₅. Note the fragment 176-192 in Table 7.
 - T at the 5th cycle indicating cleavage after V₁₇₇.
 - T at the 1st–3rd cycles, indicating increasingly common cleavages after R₁₈₁, A₁₈₀ and Y₁₇₉.
- Only the last of these correspond to peaks detected by mass spectrometry; 163-179 and 180-192, see Table 7. The absence of the others can indicate that they are on fragments smaller than were examined in the mass spectrum.
- K at the 4th, 8th, and 10th cycles indicating cleavages after E₁₈₃, Y₁₇₉, and V₁₇₇, respectively, all of which correspond to fragments observed by mass spectroscopy. See Table 7.
 - A at the 1st and 3rd cycles indicating presence of the N-terminus of the substrate and cleavage after V₁₇₇, respectively.
 - P at the 4th and 8th cycles indicating presence of the N-terminus of the substrate.

G at the 6th and 10th cycles indicating presence of the N-terminus of the substrate.

M at the 7th cycle indicating presence of the N-terminus of the substrate and/or cleavage after F₁₈₅.

M at the 15th cycle indicating cleavage after V₁₇₇.

5 The 1st cycle can indicate cleavage after D₁₉₁, see Table 7.

R at the 4th and 13th cycle indicating cleavage after V₁₇₇.

R at the 2nd and 11th cycle indicating cleavage after Y₁₇₉.

10 V at the 2nd, 6th, and 13th cycle indicating cleavage after V₁₇₅, M₁₆₉ and presence of the N-terminus of the substrate, respectively. Note fragments beginning at 176 and 170 in Table 7.

Y at the 1st, 2nd, and 14th cycles indicating cleavage after V₁₇₅, V₁₇₇, and presence of the N-terminus of the substrate, respectively.

L at the 11th and 12th cycles indicating cleavage after V₁₇₇, and presence of the N-terminus of the substrate, respectively, is the interpretation most consistent with the other data.

15 Comparing to the mass spectrometry results we see that L at the 2nd, 5th, and 9th cycles is consistent with cleavage after F₁₈₆, E₁₈₃ or M₁₆₉, and Y₁₇₉, respectively. See Table 7.

Epitope Identification

20 Fragments co-C-terminal with 8-10 amino acid long sequences predicted to bind HLA by the SYFPEITHI or NIH algorithms were chosen for further analysis. The digestion and prediction steps of the procedure can be usefully practiced in any order. Although the substrate peptide used in proteasomal digest described here was specifically designed to include a predicted HLA-A1 binding sequence, the actual products of digestion can be checked after the fact for actual or predicted binding to other MHC molecules. Selected results are shown in Table 8.

Table 8. Predicted HLA binding by proteasomally generated fragments

SEQ ID NO	PEPTIDE	HLA	SYFPEITHI	NIH
32 & (33)	(G)MPEGDLVY V	A*0201	17 (27)	(2605)
		B*0702	20	<5
		B*5101	22	314
34 & (35)	(Q)GMPEGDLV Y	A1	24 (26)	<5
		A3	16 (18)	36
		B*2705	17	25
	MPEGDLVY	B*5101	15	NP†
36	(P)EGDLVYVN Y	A1	27 (15)	12
		A26	23 (17)	NP
39	LVYVNYARTE	A3	21	<5
40 & (41)	(Y)VNYARTED F	A26	(20)	NP
		B*08	15	<5
		B*2705	12	50
42	NYARTEDFF	A24	NP†	100
		Cw*0401	NP	120
43	YARTEDFF	B*08	16	<5
44	RTEDFFKLE	A1	21	<5
		A26	15	NP

†No prediction

5 **HLA-A*0201 binding assay:**

HLA-A*0201 binding studies were performed with PSMA₁₆₈₋₁₇₇, GMPEGDLVYV, (SEQ ID NO. 33) essentially as described in Example 3 above. As seen in figure 8, this epitope exhibits significant binding at even lower concentrations than the positive control peptides. The Melan-A peptide used as a control in this assay (and throughout this disclosure), ELAGIGILTV, is actually a variant of the natural sequence (EAAGIGILTV) and exhibits a high affinity in this assay.

10

Example 5**Cluster Analysis (PSMA₂₈₁₋₃₁₀).**

Another peptide, RGIAEAVGLPSIPVHPIGYYDAQKLEKMG, PSMA₂₈₁₋₃₁₀, (SEQ ID NO. 45), containing an A1 epitope cluster from prostate specific membrane antigen, PSMA₂₈₃₋₃₀₇ (SEQ ID NO. 46), was synthesized using standard solid-phase F-moc chemistry on a 433A ABI Peptide synthesizer. After side chain deprotection and cleavage from the resin, peptide in ddH₂O was run on a reverse-phase preparative HPLC C18 column at following conditions: linear AB gradient (5% B/min) at a flow rate of 4 ml/min, where eluent A is 0.1% aqueous TFA and eluent B is 0.1% TFA in acetonitrile. A fraction at time 17.061 min containing the expected peptide as judged by mass spectrometry, was pooled and lyophilized. The peptide was then subjected to proteasome digestion and mass spectrum analysis essentially as described above. Prominent peaks from the mass spectra are summarized in Table 9.

Table 9. PSMA₂₈₁₋₃₁₀ Mass Peak Identification.

PEPTIDE	SEQUENCE	CALCULATE D MASS (MH ⁺)
281-297	RGIAEAVGLPSIPVHPI*	1727.07
286-297	AVGLPSIPVHPI**	1200.46
287-297	VGLPSIPVHPI	1129.38
288-297	GLPSIPVHPI [†]	1030.25
298-310	GYDAQKLEKMG†	1516.5
298-305	GYDAQKLS	958.05
281-305	RGIAEAVGLPSIPVHPIGYYDAQKL	2666.12
281-307	RGIAEAVGLPSIPVHPIGYYDAQKLE	2908.39
286-307	AVGLPSIPVHPIGYYDAQKLE¶	2381.78
287-307	VGLPSIPVHPIGYYDAQKLE	2310.70
288-307	GLPSIPVHPIGYYDAQKLE#	2211.57
281-299	RGIAEAVGLPSIPVHPIGY	1947
286-299	AVGLPSIPVHPIGY	1420.69
287-299	VGLPSIPVHPIGY	1349.61
288-299	GLPSIPVHPIGY	1250.48
287-310	VGLPSIPVHPIGYYDAQKLEKMG	2627.14
288-310	GLPSIPVHPIGYYDAQKLEKMG	2528.01

Boldface sequences correspond to peptides predicted to bind to MHC, see Table 10.

*By mass alone this peak could also have been 296-310 or 288-303.

**By mass alone this peak could also have been 298-307. Combination of HPLC and mass spectrometry show that at some later time points this peak is a mixture of both species.

† By mass alone this peak could also have been 289-298.

By mass alone this peak could also have been 281-295 or 294-306.

§ By mass alone this peak could also have been 297-303.

¶ By mass alone this peak could also have been 285-306.

By mass alone this peak could also have been 288-303.

5 None of these alternate assignments are supported N-terminal pool sequence analysis.

N-terminal Pool Sequence Analysis

One aliquot at one hour of the proteasomal digestion (see Example 3 part 3 above) was subjected to N-terminal amino acid sequence analysis by an ABI 473A Protein Sequencer (Applied Biosystems, Foster City, CA). Determination of the sites and efficiencies of cleavage was based on
10 consideration of the sequence cycle, the repetitive yield of the protein sequencer, and the relative yields of amino acids unique in the analyzed sequence. That is if the unique (in the analyzed sequence) residue X appears only in the nth cycle a cleavage site exists n-1 residues before it in the N-terminal direction. In addition to helping resolve any ambiguity in the assignment of mass to
15 sequences, these data also provide a more reliable indication of the relative yield of the various fragments than does mass spectrometry.

For PSMA₂₈₁₋₃₁₀ (SEQ ID NO. 45) this pool sequencing supports two major cleavage sites after V₂₈₇ and I₂₉₇ among other minor cleavage sites. Reviewing the results presented in Fig. 9 reveals the following:

20 S at the 4th and 11th cycles indicating cleavage after V₂₈₇ and presence of the N-terminus of the substrate, respectively.

H at the 8th cycle indicating cleavage after V₂₈₇. The lack of decay in peak height at positions 9 and 10 versus the drop in height present going from 10 to 11 can suggest cleavage after A₂₈₆ and E₂₈₅ as well, rather than the peaks representing latency in the
25 sequencing reaction.

D at the 2nd, 4th, and 7th cycles indicating cleavages after Y₂₉₉, I₂₉₇, and V₂₉₄, respectively.

This last cleavage is not observed in any of the fragments in Table 10 or in the alternate assignments in the notes below.

Q at the 6th cycle indicating cleavage after I₂₉₇.

30 M at the 10th and 12th cycle indicating cleavages after Y₂₉₉ and I₂₉₇, respectively.

Epitope Identification

Fragments co-C-terminal with 8-10 amino acid long sequences predicted to bind HLA by the SYFPEITHI or NIH algorithms were chosen for further study. The digestion and prediction steps of the procedure can be usefully practiced in any order. Although the substrate peptide used
35 in proteasomal digest described here was specifically designed to include a predicted HLA-A1 binding sequence, the actual products of digestion can be checked after the fact for actual or predicted binding to other MHC molecules. Selected results are shown in Table 10.

Table 10.**Predicted HLA binding by proteasomally generated fragments: PSMA₂₈₁₋₃₁₀**

SEQ ID NO.	PEPTIDE	HLA	SYFPEITHI	NIH
47 & (48)	(G) LPSIPVH PI	A*0201	16 (24)	(24)
		B*0702/B7	23	12
		B*5101	24	572
		Cw*0401	NP†	20
49 & (50)	(P) IGYDAQ KL	A*0201	(16)	<5
		A26	(20)	NP
		B*2705	16	25
		B*2709	15	NP
		B*5101	21	57
		Cw*0301	NP	24
51 & (52)	(P) SIPVHPI GY	A1	21 (27)	<5
		A26	22	NP
		A3	16	<5
		B*5101	16	NP
53	IPVHPIGY			
54	YYDAQKLE	A1	22	<5

†No prediction

5

As seen in Table 10, N-terminal addition of authentic sequence to epitopes can often generate still useful, even better epitopes, for the same or different MHC restriction elements. Note for example the pairing of (G)LPSIPVHPI with HLA-A*0201, where the 10-mer can be used as a vaccine useful with several MHC types by relying on N-terminal trimming to create the epitopes for HLA-B7, -B*5101, and Cw*0401.

10

HLA-A*0201 binding assay:

HLA-A*0201 binding studies were preformed with PSMA₂₈₈₋₂₉₇, GLPSIPVHPI, (SEQ ID NO. 48) essentially as described in Examples 3 and 4 above. As seen in figure 8, this epitope exhibits significant binding at even lower concentrations than the positive control peptides.

Example 6**Cluster Analysis (PSMA₄₅₄₋₄₈₁).**

Another peptide, SSIEGNYTLRV DCTPLMYSLVHLTKEL, PSMA₄₅₄₋₄₈₁, (SEQ ID NO. 55) containing an epitope cluster from prostate specific membrane antigen, was synthesized by MPS (purity >95%) and subjected to proteasome digestion and mass spectrum analysis as described above. Prominent peaks from the mass spectra are summarized in Table 11.

Table 11. PSMA₄₅₄₋₄₈₁ Mass Peak Identification.

MS PEAK (measured)	PEPTIDE	SEQUENCE	CALCULATED MASS (MH ⁺)
1238.5	454-464	SSIEGNYTLRV	1239.78
1768.38±0.60	454-469	SSIEGNYTLRV DCTPL	1768.99
1899.8	454-470	SSIEGNYTLRV DCTPLM	1900.19
1097.63±0.91	463-471	RVDCTPLMY	1098.32
2062.87±0.68	454-471*	SSIEGNYTLRV DCTPLMY	2063.36
1153	472-481**	SLVHNLTKEL	1154.36
1449.93±1.79	470-481	MYSLVHNLTKEL	1448.73

Boldface sequence correspond to peptides predicted to bind to MHC, see Table 12.

* On the basis of mass alone this peak could equally well be assigned to the peptide 455-472 however proteasomal removal of just the N-terminal amino acid is considered unlikely. If the issue were important it could be resolved by N-terminal sequencing.

**On the basis of mass this fragment might also represent 455-464.

Epitope Identification

Fragments co-C-terminal with 8-10 amino acid long sequences predicted to bind HLA by the SYFPEITHI or NIH algorithms were chosen for further study. The digestion and prediction steps of the procedure can be usefully practiced in any order. Although the substrate peptide used in proteasomal digest described here was specifically designed to include predicted HLA-A2.1 binding sequences, the actual products of digestion can be checked after the fact for actual or predicted binding to other MHC molecules. Selected results are shown in Table 12.

Table 12. Predicted HLA binding by proteasomally generated fragments

<u>SEQ ID NO</u>	<u>PEPTIDE</u>	<u>HLA</u>	<u>SYFPEITHI</u>	<u>NIH</u>
56 & (57)	(S) IEGNYTLRV	A1	(19)	<5
58	EGNYTLRV	A*0201	16 (22)	<5
		B*5101	15	NP†
59 & (60)	(Y) TLRVDCTPL	A*0201	20 (18)	(5)
		A26	16 (18)	NP
		B7	14	40
		B8	23	<5
		B*2705	12	30
		Cw*0301	NP	(30)
61	LRVDCTPLM	B*2705	20	600
		B*2709	20	NP
62 & (63)	(L) RVDCTPLMY	A1	32 (22)	125 (13.5)
		A3	25	<5
		A26	22	NP
		B*2702	NP	(200)
		B*2705	13 (NP)	(1000)

†No prediction

5 As seen in Table 12, N-terminal addition of authentic sequence to epitopes can often generate still useful, even better epitopes, for the same or different MHC restriction elements. Note for example the pairing of (L)RVDCTPLMY (SEQ ID NOS 62 and (63)) with HLA-B*2702/5, where the 10-mer has substantial predicted halftimes of dissociation and the co-C-terminal 9-mer does not. Also note the case of SIEGNYTLRV (SEQ ID NO 57) a predicted HLA-A*0201 epitope which can be used as a vaccine useful with HLA-B*5101 by relying on N-terminal trimming to create the epitope.

HLA-A*0201 binding assay

15 HLA-A*0201 binding studies were preformed, essentially as described in Example 3 above, with PSMA₄₆₀₋₄₆₉, TLRVDCTPL, (SEQ ID NO. 60). As seen in figure 10, this epitope was found to bind HLA-A2.1 to a similar extent as the known A2.1 binder FLPSDYFPSV (HBV₁₈₋₂₇; SEQ ID NO: 24) used as a positive control. Additionally, PSMA₄₆₁₋₄₆₉, (SEQ ID NO. 59) binds nearly as well.

ELISPOT analysis: PSMA₄₆₃₋₄₇₁ (SEQ ID NO. 62)

20 The wells of a nitrocellulose-backed microtiter plate were coated with capture antibody by incubating overnight at 4°C using 50 µl/well of 4µg/ml murine anti-human γ-IFN monoclonal

antibody in coating buffer (35 mM sodium bicarbonate, 15 mM sodium carbonate, pH 9.5). Unbound antibody was removed by washing 4 times 5 min. with PBS. Unbound sites on the membrane then were blocked by adding 200 μ l/well of RPMI medium with 10% serum and incubating 1 hr. at room temperature. Antigen stimulated CD8⁺ T cells, in 1:3 serial dilutions, were seeded into the wells of the microtiter plate using 100 μ l/well, starting at 2x10⁵ cells/well. (Prior antigen stimulation was essentially as described in Scheibenbogen, C. et al. *Int. J. Cancer* 71:932-936, 1997. PSMA₄₆₂₋₄₇₁ (SEQ ID NO. 62) was added to a final concentration of 10 μ g/ml and IL-2 to 100 U/ml and the cells cultured at 37°C in a 5% CO₂, water-saturated atmosphere for 40 hrs. Following this incubation the plates were washed with 6 times 200 μ l/well of PBS containing 0.05% Tween-20 (PBS-Tween). Detection antibody, 50 μ l/well of 2g/ml biotinylated murine anti-human γ -IFN monoclonal antibody in PBS+10% fetal calf serum, was added and the plate incubated at room temperature for 2 hrs. Unbound detection antibody was removed by washing with 4 times 200 μ l of PBS-Tween. 100 μ l of avidin-conjugated horseradish peroxidase (Pharmingen, San Diego, CA) was added to each well and incubated at room temperature for 1 hr. Unbound enzyme was removed by washing with 6 times 200 μ l of PBS-Tween. Substrate was prepared by dissolving a 20 mg tablet of 3-amino 9-ethylcarbasole in 2.5 ml of N, N-dimethylformamide and adding that solution to 47.5 ml of 0.05 M phosphate-citrate buffer (pH 5.0). 25 μ l of 30% H₂O₂ was added to the substrate solution immediately before distributing substrate at 100 μ l/well and incubating the plate at room temperature. After color development (generally 15-30 min.), the reaction was stopped by washing the plate with water. The plate was air dried and the spots counted using a stereomicroscope.

Figure 11 shows the detection of PSMA₄₆₃₋₄₇₁ (SEQ ID NO. 62)-reactive HLA-A1⁺ CD8⁺ T cells previously generated in cultures of HLA-A1⁺ CD8⁺ T cells with autologous dendritic cells plus the peptide. No reactivity is detected from cultures without peptide (data not shown). In this case it can be seen that the peptide reactive T cells are present in the culture at a frequency between 1 in 2.2x10⁴ and 1 in 6.7x10⁴. That this is truly an HLA-A1-restricted response is demonstrated by the ability of anti-HLA-A1 monoclonal antibody to block γ -IFN production; see figure 12.

Example 7

Cluster Analysis (PSMA₆₅₃₋₆₈₇).

Another peptide, FDKSNPIVLRMMNDQLMFLERAFIDPLGLPDRPFY PSMA₆₅₃₋₆₈₇, (SEQ ID NO. 64) containing an A2 epitope cluster from prostate specific membrane antigen, PSMA₆₆₀₋₆₈₁ (SEQ ID NO 65), was synthesized by MPS (purity >95%) and subjected to proteasome digestion and mass spectrum analysis as described above. Prominent peaks from the mass spectra are summarized in Table 13.

Table 13. PSMA₆₅₃₋₆₈₇ Mass Peak Identification.

MS PEAK (measured)	PEPTIDE	SEQUENCE	CALCULATED MASS (MH ⁺)
906.17±0.65	681-687**	LPDRPFY	908.05
1287.73±0.76	677-687**	DPLGLPDRPFY	1290.47
1400.3±1.79	676-687	IDPLGLPDRPFY	1403.63
1548.0±1.37	675-687	FIDPLGLPDRPFY	1550.80
1619.5±1.51	674-687**	AFIDPLGLPDRPFY	1621.88
1775.48±1.32	673-687*	RAFIDPLGLPDRPFY	1778.07
2440.2±1.3	653-672	FDKSNPIVLRMMNDQLMFLE	2442.932
1904.63±1.56	672-687*	ERAFIDPLGLPDRPFY	1907.19
2310.6±2.5	653-671	FDKSNPIVLRMMNDQLMFL	2313.82
2017.4±1.94	671-687	LERAFIDPLGLPDRPFY	2020.35
2197.43±1.78	653-670	FDKSNPIVLRMMNDQLMF	2200.66

Boldface sequence correspond to peptides predicted to bind to MHC, see Table 13.

* On the basis of mass alone this peak could equally well be assigned to a peptide beginning at 654, however proteasomal removal of just the N-terminal amino acid is considered unlikely. If the issue were important it could be resolved by N-terminal sequencing.

** On the basis of mass alone these peaks could have been assigned to internal fragments, but given the overall pattern of digestion it was considered unlikely.

Epitope Identification

Fragments co-C-terminal with 8-10 amino acid long sequences predicted to bind HLA by the SYFPEITHI or NIH algorithms were chosen for further study. The digestion and prediction steps of the procedure can be usefully practiced in any order. Although the substrate peptide used in proteasomal digest described here was specifically designed to include predicted HLA-A2.1 binding sequences, the actual products of digestion can be checked after the fact for actual or predicted binding to other MHC molecules. Selected results are shown in Table 14.

Table 14. Predicted HLA binding by proteasomally generated fragments

SEQ ID NO	PEPTIDE	HLA	SYFPEITHI	NIH
66 & (67)	(R)MMNDQLMF L	A*0201	24 (23)	1360 (722)
		A*0205	NP†	71 (42)
		A26	15	NP
		B*2705	12	50
68	RMMNDQLMF	B*2705	17	75

†No prediction

5

As seen in Table 14, N-terminal addition of authentic sequence to epitopes can generate still useful, even better epitopes, for the same or different MHC restriction elements. Note for example the pairing of (R)MMNDQLMFL (SEQ ID NOS. 66 and (67)) with HLA-A*02, where the 10-mer retains substantial predicted binding potential.

10

HLA-A*0201 binding assay

HLA-A*0201 binding studies were performed, essentially as described in Example 3 above, with PSMA₆₆₃₋₆₇₁, (SEQ ID NO. 66) and PSMA₆₆₂₋₆₇₁, RMMNDQLMFL (SEQ NO. 67). As seen in figures 10, 13 and 14, this epitope exhibits significant binding at even lower concentrations than the positive control peptide (FLPSDYFPSV (HBV₁₈₋₂₇); SEQ ID NO: 24). Though not run in parallel, comparison to the controls suggests that PSMA₆₆₂₋₆₇₁ (which approaches the Melan A peptide in affinity) has the superior binding activity of these two PSMA peptides.

15

Example 8**Vaccinating with epitope vaccines.**

20

1. Vaccination with peptide vaccines:A. Intranodal delivery

A formulation containing peptide in aqueous buffer with an antimicrobial agent, an antioxidant, and an immunomodulating cytokine, was injected continuously over several days into the inguinal lymph node using a miniature pumping system developed for insulin delivery (MiniMed; Northridge, CA). This infusion cycle was selected in order to mimic the kinetics of antigen presentation during a natural infection.

25

B. Controlled release

A peptide formulation is delivered using controlled PLGA microspheres as is known in the art, which alter the pharmacokinetics of the peptide and improve immunogenicity. This formulation is injected or taken orally.

30

C. Gene gun delivery

A peptide formulation is prepared wherein the peptide is adhered to gold microparticles as is known in the art. The particles are delivered in a gene gun, being accelerated at high speed so as to penetrate the skin, carrying the particles into dermal tissues that contain pAPCs.

5 D. Aerosol delivery

A peptide formulation is inhaled as an aerosol as is known in the art, for uptake into appropriate vascular or lymphatic tissue in the lungs.

2. Vaccination with nucleic acid vaccines:

10 A nucleic acid vaccine is injected into a lymph node using a miniature pumping system, such as the MiniMed insulin pump. A nucleic acid construct formulated in an aqueous buffered solution containing an antimicrobial agent, an antioxidant, and an immunomodulating cytokine, is delivered over a several day infusion cycle in order to mimic the kinetics of antigen presentation during a natural infection.

15 Optionally, the nucleic acid construct is delivered using controlled release substances, such as PLGA microspheres or other biodegradable substances. These substances are injected or taken orally. Nucleic acid vaccines are given using oral delivery, priming the immune response through uptake into GALT tissues. Alternatively, the nucleic acid vaccines are delivered using a gene gun, wherein the nucleic acid vaccine is adhered to minute gold particles. Nucleic acid constructs can also be inhaled as an aerosol, for uptake into appropriate vascular or lymphatic tissue in the lungs.

20 **Example 9**

Assays for the effectiveness of epitope vaccines.

1. Tetramer analysis:

25 Class I tetramer analysis is used to determine T cell frequency in an animal before and after administration of a housekeeping epitope. Clonal expansion of T cells in response to an epitope indicates that the epitope is presented to T cells by pAPCs. The specific T cell frequency is measured against the housekeeping epitope before and after administration of the epitope to an animal, to determine if the epitope is present on pAPCs. An increase in frequency of T cells specific to the epitope after administration indicates that the epitope was presented on pAPC.

2. Proliferation assay:

30 Approximately 24 hours after vaccination of an animal with housekeeping epitope, pAPCs are harvested from PBMCs, splenocytes, or lymph node cells, using monoclonal antibodies against specific markers present on pAPCs, fixed to magnetic beads for affinity purification. Crude blood or splenocyte preparation is enriched for pAPCs using this technique. The enriched pAPCs are then used in a proliferation assay against a T cell clone that has been generated and is specific for
35 the housekeeping epitope of interest. The pAPCs are coincubated with the T cell clone and the T cells are monitored for proliferation activity by measuring the incorporation of radiolabeled

thymidine by T cells. Proliferation indicates that T cells specific for the housekeeping epitope are being stimulated by that epitope on the pAPCs.

3. Chromium release assay:

5 A human patient, or non-human animal genetically engineered to express human class I MHC, is immunized using a housekeeping epitope. T cells from the immunized subject are used in a standard chromium release assay using human tumor targets or targets engineered to express the same class I MHC. T cell killing of the targets indicates that stimulation of T cells in a patient would be effective at killing a tumor expressing a similar TuAA.

Example 10

10 **Induction of CTL response with naked DNA is efficient by Intra-lymph node immunization.**

In order to quantitatively compare the CD8⁺ CTL responses induced by different routes of immunization a plasmid DNA vaccine (pEGFP33A) containing a well-characterized immunodominant CTL epitope from the LCMV-glycoprotein (G) (gp33; amino acids 33-41) (Oehen, S., et al., *Immunology* 99, 163-169 2000) was used, as this system allows a comprehensive
15 assessment of antiviral CTL responses. Groups of 2 C57BL/6 mice were immunized once with titrated doses (200-0.02μg) of pEGFP33A DNA or of control plasmid pEGFP-N3, administered i.m. (intramuscular), i.d. (intradermal), i.spl. (intrasplenic), or i.ln. (intra-lymph node). Positive control mice received 500 pfu LCMV i.v. (intravenous). Ten days after immunization spleen cells were isolated and gp33-specific CTL activity was determined after secondary *in vitro*
20 restimulation. As shown in Fig. 15, i.m. or i.d. immunization induced weakly detectable CTL responses when high doses of pEGFP33A DNA (200μg) were administered. In contrast, potent gp33-specific CTL responses were elicited by immunization with only 2μg pEGFP33A DNA i.spl. and with as little as 0.2μg pEGFP33A DNA given i.ln. (figure 15; symbols represent individual mice and one of three similar experiments is shown). Immunization with the control
25 pEGFP-N3 DNA did not elicit any detectable gp33-specific CTL responses (data not shown).

Example 11

Intra-lymph node DNA immunization elicits anti-tumor immunity.

To examine whether the potent CTL responses elicited following i.ln. immunization were able to confer protection against peripheral tumors, groups of 6 C57BL/6mice were immunized
30 three times at 6-day intervals with 10μg of pEGFP33A DNA or control pEGFP-N3 DNA. Five days after the last immunization small pieces of solid tumors expressing the gp33 epitope (EL4-33) were transplanted s.c. into both flanks and tumor growth was measured every 3-4d. Although the EL4-33 tumors grew well in mice that had been repetitively immunized with control pEGFP-N3 DNA (figure 16), mice which were immunized with pEGFP33A DNA i.ln. rapidly eradicated the
35 peripheral EL4-33 tumors (figure 16).

Example 12

Differences in lymph node DNA content mirrors differences in CTL response following intra-lymph node and intramuscular injection.

pEFGPL33A DNA was injected i.ln. or i.m. and plasmid content of the injected or draining lymph node was assessed by real time PCR after 6, 12, 24, 48 hours, and 4 and 30 days. At 6, 12, and 24 hours the plasmid DNA content of the injected lymph nodes was approximately three orders of magnitude greater than that of the draining lymph nodes following i.m. injection. No plasmid DNA was detectable in the draining lymph node at subsequent time points (Fig. 17). This is consonant with the three orders of magnitude greater dose needed using i.m. as compared to i.ln. injections to achieve a similar levels of CTL activity. CD8⁺ knockout mice, which do not develop a CTL response to this epitope, were also injected i.ln. showing clearance of DNA from the lymph node is not due to CD8⁺ CTL killing of cells in the lymph node. This observation also supports the conclusion that i.ln. administration will not provoke immunopathological damage to the lymph node.

Example 13

Administration of a DNA plasmid formulation of a therapeutic vaccine for melanoma to humans.

SYNCHROTOPE TA2M, a melanoma vaccine, encoding the HLA-A2-restricted tyrosinase epitope SEQ ID NO. 1 and epitope cluster SEQ ID NO. 69, was formulated in 1% Benzyl alcohol, 1% ethyl alcohol, 0.5mM EDTA, citrate-phosphate, pH 7.6. Aliquots of 80, 160, and 320 µg DNA/ml were prepared for loading into MINIMED 407C infusion pumps. The catheter of a SILHOUETTE infusion set was placed into an inguinal lymph node visualized by ultrasound imaging. The assembly of pump and infusion set was originally designed for the delivery of insulin to diabetics and the usual 17mm catheter was substituted with a 31mm catheter for this application. The infusion set was kept patent for 4 days (approximately 96 hours) with an infusion rate of about 25 µl/hour resulting in a total infused volume of approximately 2.4 ml. Thus the total administered dose per infusion was approximately 200, and 400 µg; and can be 800 µg, respectively, for the three concentrations described above. Following an infusion subjects were given a 10 day rest period before starting a subsequent infusion. Given the continued residency of plasmid DNA in the lymph node after administration (as in example 12) and the usual kinetics of CTL response following disappearance of antigen, this schedule will be sufficient to maintain the immunologic CTL response.

Example 14**Additional Epitopes.**

The methodologies described above, and in particular in examples 3-7, have been applied to additional synthetic peptide substrates, leading to the identification of further epitopes as set for the in tables 15-36 below. The substrates used here were designed to identify products of housekeeping proteasomal processing that give rise to HLA-A*0201 binding epitopes, but additional MHC-binding reactivities can be predicted, as discussed above. Many such reactivities are disclosed, however, these listings are meant to be exemplary, not exhaustive or limiting. As also discussed above, individual components of the analyses can be used in varying combinations and orders. The digests of the NY-ESO-1 substrates 136-163 and 150-177 (SEQ ID NOS. 254 and 255, respectively) yielded fragments that did not fly well in MALDI-TOF mass spectrometry. However, they were quite amenable to N-terminal peptide pool sequencing, thereby allowing identification of cleavage sites. Not all of the substrates necessarily meet the formal definition of an epitope cluster as referenced in example 3. Some clusters are so large, e.g. NY-ESO-1₈₆₋₁₇₁, that it was more convenient to use substrates spanning only a portion of this cluster. In other cases, substrates were extended beyond clusters meeting the formal definition to include neighboring predicted epitopes. In some instances, actual binding activity may have dictated what substrate was made, as with for example the MAGE epitopes reported here, where HLA binding activity was determined for a selection of peptides with predicted affinity, before synthetic substrates were designed.

Table 15

GP100: Preferred Epitopes Revealed by Houskeeping Proteasome Digestion

†Scores are given from the two binding prediction programs referenced above (see example 3).

Substrate	Epitope	Sequence	SEQ ID NO	HLA Binding Predictions (SYFPEITHI /NIH)†					Comments
				A*0201	A1	A3	B7	B8	
609-644	630-638*	LPHSSSHWL	88				20/80	16/<5	*The digestion of 609-644 and 622-650 have generated the same epitopes.
	629-638*	QLPHSSSHWL	89	21/117					
	614-622	LIYRRRLMK	90			32/20			
	613-622	SLIYRRRLMK	91	14/<5		29/60			
	615-622	IYRRRLMK	92					15/<5	
622-650	630-638*	LPHSSSHWL	93				20/80	16/<5	
	629-638*	QLPHSSSHWL	94	21/117					

Table 16A

MAGE-1: Preferred Epitopes Revealed by Housekeeping Proteasome Digestion

HLA Binding Predictions (SYFPEITHI/NIH) [†]									
Substrate	Epitope	Sequence	SEQ ID NO	A*0201	A1	A3	B7	B8	Other
86-109	95-102	ESLFRAVI	95					16/<5	
	93-102	ILESFRAVI	96	21/<5		20/<5			
	93-101	ILESFRAV	97	23/<5					
	92-101	CILESFRAV	98	23/55					
	92-100	CILESFR	99	20/138					
	263-271	EFLWGPRAL	100						A26 (R 21), A24 (NIH 30)
263-292	264-271	FLWGPRAL	101					17/<5	
	264-273	FLWGPRALAE	102	16/<5		19/<5			
	265-274	LWGPRALAE	103	16/<5					
	268-276	PRALAEYS	104	15/<5					
	267-276	GPRALAEYS	105	15/<5			<15/<5		B4403 (NIH 7); B3501 (NIH 120)
	269-277	RALAEYSV	106	18/20					
	271-279	LAETSYVKV	107	19/<5					
	270-279	ALAETSYVKV	108	30/427		19/<5<5			
	272-280	AETSYVKVL	109	15/<5					B4403 (NIH 36)
	271-280	LAETSYVKVL	110	18/<5			<15/<5		
	274-282	TSYVKVLEY	111		26/<5				B4403 (NIH 14)
	273-282	ETSYVKVLEY	112		28/6				A26 (R 31), B4403 (NIH 14)
	278-286	KVI FVYVKV	113	26/743			16/<5		

Table 16B
MAGE-1: Preferred Epitopes Revealed by Housekeeping Proteasome Digestion

Substrate	Epitope	Sequence	SEQ ID NO	HLA Binding Predictions (SYFPEITHI/NIH) [†]					
				A*0201	A1	A3	B7	B8	Other
168-193	168-177	SYVLVTCGL	114						A24 (NIH 300)
	169-177	YVLVTCGL	115	20/32		15/<5	<15/20		
	170-177	VLVTCGL	116					17/<5	
	240-248	TQDLVQEKY	117		29/<5				
229-258	239-248	LTQDLVQEKY	118		23/<5				A26 (R 22)
	232-240	YGEPRKLLT	119		24/11				
	243-251	LVQEKYLEY	120		21/<5	21/<5			A26 (R 28)
	242-251	DLVQEKYLEY	121		22/<5	19/<5			A26 (R 30)
	230-238	SAYGEPRKL	122	21/<5					B5101 (25/121)
	278-286	KVLEYVIKV	123	26/743		16/<5			
	277-286	VKVLEYVIKV	124	17/<5					
	276-284	YVKVLEYVI	125	15/<5		15/<5		17/<5	
272-297	274-282	TSYVKVLEY	126		26/<5				
	273-282	ETSYVKVLEY	127		28/6				
	283-291	VIKVSARVR	128			20/<5			
	282-291	YVIKVSARVR	129			24/<5			

[†]Scores are given from the two binding prediction programs referenced above (see example 3). R indicates a SYFPEITHI score.

Table 17A

MAGE-2: Preferred Epitopes Revealed by Housekeeping Proteasome Digestion

MAGE-2: Preferred Epitopes Revealed by Housekeeping Proteasome Digestion									
Substrate	Epitope	Sequence	SEQ ID NO	HLA Binding Predictions (SYFPEITHI/NIH) [†]					
				A*0201	A1	A3	B7	B8	Other
107-126	115-122	ELVHFLLL	130					18/<5	
	113-122	MVELYHFLLL	131		21/<5				A26 (R 22)
	109-116	ISRKMVEL	132					17/<5	
	108-116	AISRKMVEL	133	25/7			16/12	26/<5	
	107-116	AISRKMVEL	134	22/<5		19/<5	14/36	n.p./16	
	112-120	KMVELVHFL	135	27/2800					
	109-117	ISRKMVELV	136	16/<5					
	108-117	AISRKMVELV	137	24/11					
	116-124	LVHFLLLY	138		23/<5	19/<5			A26 (R 26)
	115-124	ELVHFLLLY	139		24/<5	19/5			A26 (R 29)
	111-119	RKMVELVHF	140						
	158-166	LQLVFGIEV	141	17/168					
	157-166	YLQLVFGIEV	142	24/1215					
	159-167	QLVFGIEVV	143	25/32		18/<5			
145-175	158-167	LQLVFGIEVV	144	18/20					
	164-172	IEVVEVPI	145	16/<5					
	163-172	GIEVVEVPI	146	22/<5					
	162-170	FGIEVVEVV	147	19/<5					B5101(24/69.212)
	154-162	ASEYLQLVF	148		22/68				
	153-162	KASEYLQLVF	149			15/<5			

[†]Scores are given from the two binding prediction programs referenced above (see example 3). R indicates a SYFPEITHI score.

Table 17B
MAGE-2: Preferred Epitopes Revealed by Housekeeping Proteasome Digestion

Substrate	Epitope	Sequence		HLA Binding Predictions (SYFPEITHI /NIH) [†]					
				A*0201	A1	A3	B7	B8	Other
213-233	218-225	EEKIWEEL	150					22/<5	
	216-225	APEEKIWEEL	151	15/<5			22/72		
	216-223	APEEKIWE	152					18/<5	
	220-228	KIWEELSML	153	26/804		16/<5		16/<5	A26 (R 26)
	219-228	EKIWEELSML	154						A26 (R 22)
	271-278	FLWGPRAL	155					17/<5	
271-291	271-279	FLWGPRALI	156	25/398		16/7			
	278-286	LIETSYVKV	157	23/<5					
	277-286	ALIETSYVKV	158	30/427		21/<5			
	276-284	RALIETSYV	159	18/19					B5101 (20/55)
	279-287	IETSYVKVL	160	15/<5					
	278-287	LIETSYVKVL	161	22/<5					A26 (R 22)

[†]Scores are given from the two binding prediction programs referenced above (see example 3). R indicates a SYFPEITHI score.

Table 18
MAGE-3: Preferred Epitopes Revealed by Housekeeping Proteasome Digestion

Substrate	Epitope	Sequence	SEQ ID NO	HLA Binding Predictions (SYFPEITHI/NIH) [†]					
				A*0201	A1	A3	B7	B8	Other
267-286	271-278	FLWGPRAL	162					17/<5	
	270-278	EFLWGPRAL	163						A26 (R 21); A24 (NIH 30)
	271-279	FLWGPRALV	164	27/2655		16/<5			
	276-284	RALVETSYV	165	18/19					B5101 (20/55)
	272-280	LWGPRALVE	166			15/<5			
	271-280	FLWGPRALVE	167	15/<5		22/<5			
	272-281	LWGPRALVET	168	16/<5					

[†]Scores are given from the two binding prediction programs referenced above (see example 3). R indicates a SYFPEITHI score.

Table 19A
 NY-ESO-1: Preferred Epitopes Revealed by Housekeeping Proteasome Digestion

Substrate	Epitope	Sequence	SEQ ID NO	HLA Binding Predictions (SYFPEITHI/NIH) [†]					Other
				A*0201	A1	A3	B7	B8	
81-113	82-90	GPESRLLEF	169		16/11		18/<5	22/<5	
	83-91	PESRLLEFY	170		15/<5				B4403 (NIH 18)
	82-91	GPESRLLEFY	171		25/11				
	84-92	ESRLLEFY	172					19/8	
	86-94	RLLEFYLAM	173	21/430		21/<5			
	88-96	LEFYLAMPF	174						B4403 (NIH 60)
	87-96	LLEFYLAMPF	175		<15/45	18/<5			
	93-102	AMPFATPMEA	176	15/<5					
	94-102	MPFATPMEA	177				17/<5		
	115-123	PLPVPGVLL	178	20/<5		17/<5	16/<5	18/<5	
101-133	114-123	PPLPVPGVLL	179				23/12		
	116-123*	LPVPGVLL	180					16/<5	*Evidence of the same epitope obtained from two digests.
	103-112	ELARRSLAQD	181	15/<5		20/<5			
	118-126*	VPGVLLKEF	182				17/<5	16/<5	
	117-126*	PVPGVLLKEF	183			16/<5			
	116-123*	LPVPGVLL	184					16/<5	
	127-135	TVSGNILTI	185	21/<5		19/<5			
	126-135	FTVSGNILTI	186	20/<5					
	120-128	GVLKLEFTV	187	20/130		18/<5			
	121-130	VLLKLEFTVSG	188	17/<5		18/<5			
122-130	LLKLEFTVSG	189	20/<5		18/<5				
116-145	118-126*	VPGVLLKEF	190				17/<5	16/<5	
	117-126*	PVPGVLLKEF	191			16/<5			

[†]Scores are given from the two binding prediction programs referenced above (see example 3).

Table 19B
NY-ESO-1: Preferred Epitopes Revealed by Housekeeping Proteasome Digestion

Substrate	Epitope	Sequence	SEQ ID NO	HLA Binding Predictions (SYFPEITHI/NIB) [†]					
				A*0201	A1	A3	B7	B8	Other
136-163 (SEQ ID NO 254)	139-147	AADHRQLQL	192	17/<5	17/<5			22/<5	
	148-156	SISSCLQQL	193	24/7					A26 (R 25)
	147-156	LSISSCLQQL	194	18/<5					
	138-147	TAADHRQLQL	195	18/<5					
	161-169	WITQCFLPV	196	18/84					
150-177 (SEQ ID NO 255)	157-165	SLLMWITQC	197	18/42		17/<5			
	150-158	SSCLQQLSL	198	15/<5					
	154-162	QQLSLLMWI	199	15/50					
	151-159	SCLQQLSLL	200	18/<5					
	150-159	SSCLQQLSLL	201	16/<5					
	163-171	TQCFLPVFL	202	<15/12					
	162-171	ITQCFLPVFL	203	18/<5					A26 (R 19)

[†]Scores are given from the two binding prediction programs referenced above (see example 3). R indicates a SYFPEITHI score

Table 20
PRAME: Preferred Epitopes Revealed by Housekeeping Proteasome Digestion

Substrate	Epitope	Sequence	SEQ ID NO	HLA Binding Predictions (SYFPEITHI/NIH) [†]					
				A*0201	A1	A3	B7	B8	Other
211-245	219-227	PMQDIKMIL	204	16/<5				16/n.d.	A26 (R 20)
	218-227	MPMQDIKMIL	205				<15/240		
411-446	428-436	QHLIGLSNL	206	18/<5					
	427-436	LQHLIGLSNL	207	16/8					
	429-436	HLIGLSNL	208					17/<5	B15 (R 21)
	431-439	IGLSNLTHV	209	18/7					B*5101 (R 22)
	430-439	LIGLSNLTHV	210	24/37					

[†]Scores are given from the two binding prediction programs referenced above (see example 3). R indicates a SYFPEITHI score.

Table 21
PSA: Preferred Epitopes Revealed by Housekeeping Proteasome Digestion

Substrate	Epitope	Sequence	SEQ ID NO	HLA Binding Predictions (SYFPEITHI/NID)†					
				A*0201	A1	A3	B7	B8	Other
42-77	53-61	VLVHPQWVL	211	22/112			<15/6	17/≤5	
	52-61	GVLVHPQWVL	212	17/21		16/≤5	<15/30		A26 (R 18)
	52-60	GVLVHPQWV	213	17/124					
	59-67	WVLTAHAI	214	15/16					
	54-63	LVHPQWVLT	215	19/≤5		20/≤5			A26 (R 16)
	53-62	VLVHPQWVLT	216	17/22					
	54-62	LVHPQWVLT	217			17/n.d.			
55-95	66-73	CIRNKSVI	218					26/20	
	65-73	HCIRNKSVI	219					<15/16	
	56-64	HPQWVLTAA	220				18/≤5		
	63-72	AAHCIRNKS	221	17/≤5					

†Scores are given from the two binding prediction programs referenced above (see example 3). R indicates a SYFPEITHI score.

Table 22
PSCA: Preferred Epitopes Revealed by Housekeeping Proteasome Digestion

Substrate	Epitope	Sequence	SEQ ID NO	HLA Binding Predictions (SYFPEITHI /NIB)†					
				A*0201	A1	A3	B7	B8	Other
93-123*	116-123	LLWGPQQL	222					16/<5	
	115-123	LLLWGPQQL	223	<15/18					
	114-123	GLLLWGPQQL	224	<15/10					
	99-107	ALQPAAAIL	225	26/9		22/<5	<15/12	16/<5	A26 (R 19)
	98-107	HALQPAAAIL	226	18/<5			<15/12		

*L123 is the C-terminus of the natural protein.

†Scores are given from the two binding prediction programs referenced above (see example 3).

Table 23
Tyrosinase: Preferred Epitopes Revealed by Housekeeping Proteasome Digestion

Substrate	Epitope	Sequence	SEQ ID NO	HLA Binding Predictions (SYFPEITHI/NIH) [†]					
				A*0201	A1	A3	B7	B8	Other
128-157	128-137	APEKDKFFAY	227		29/6		15/<5		B4403 (NIH 14)
	129-137	PEKDKFFAY	228		18/<5			21/<5	
	130-138	EKDKFFAYL	229				15/<5		
	131-138	KDKFFAYL	230					20/<5	
197-228	205-213	PAFLPWHL	231					15/<5	
	204-213	APAFLPWHL	232				23/360		
	207-216	FLPWHLFL	1	25/1310				<15/8	
	208-216	LPWHLFL	9	17/26		15/<5	20/80	24/16	
	214-223	FLRWQEIQ	233			16/<5			
	212-220	RLFLLRWEQ	234						
191-211	191-200	GSEIWRDIDF	235		18/68				B4403 (NIH 400)
	192-200	SEIWRDIDF	236					16/<5	
207-230	207-215	FLWHLFL	8	22/540			<15/6	17/<5	
466-484	473-481	RIWSWLLGA	237	19/13		15/<5			
476-497	476-484	SWLLGAAMV	238	18/<5					
	477-486	WLLGAAMVGA	239	21/194		18/<5			
	478-486	LLGAAMVGA	240	19/19		16/<5			

[†]Scores are given from the two binding prediction programs referenced above (see example 3).

Table 24
PSMA: Preferred Epitopes Revealed by Housekeeping Proteasome Digestion

Substrate	Epitope	Sequence	SEQ ID NO	HLA Binding Predictions (SYFPEITHI /NIB) [†]						
				A*0201	A1	A3	B7	B8	Other	
1-30	4-12	LLHETDSAV	241	25/485		15/<5				
	13-21	ATARRPRWL	242	18/<5				18/<5	A26 (R 19)	
	53-61	TPKHNNMKAF	243					24/<5		
53-80	64-73	ELKAENIKKF	244			17/<5			A26 (R 30)	
	69-77	NIKKFLH ¹ NF	245						A26 (R 27)	
	68-77	ENIKKFLH ¹ NF	246						A26 (R 24)	
215-244	220-228	AGAKGVILY	247		25/<5					
457-489	468-477	PLMYSLVHNL	248	22/<5						
	469-477	LMYSLVHNL	249	27/193		<15/9				
	463-471	RVDCTPLMY	250		32/125	25/<5			A26 (R 22)	
	465-473	DCTPLMYSL	251						A26 (R 22)	
503-533	507-515	SGMPRISKL	252	21/<5				21/<5		
	506-515	FSGMPRISKL	253	17/<5						

¹This H was reported as Y in the SWISSPROT database.

[†]Scores are given from the two binding prediction programs referenced above (see example 3).

Table 25A
MAGE-1: Preferred Epitopes Revealed by Housekeeping Proteasome Digestion

Substrate	Epitope	Sequence	Seq. ID No.	Binding Prediction		
				HLA type	SYFPEITHI	NIH
Mage-1 119-146	125-132	KAEMLESV	256	B5101	19	n.a.
	124-132	TKAEMLESV	257	A0201	20	<5
	123-132	VTKAEMLESV	258	A0201	20	<5
	128-136	MLESVIKNY	259	A1	28	45
				A26	24	n.a.
				A3	17	5
				A1	15	<1.0
	127-136	EMLESVIKNY	260	A26	23	<1.0
	125-133	KAEMLESVI	261	B5101	23	100
				A24	N.A.	4
Mage-1 143-170	146-153	KASESLQL	262	B08	16	<1.0
	145-153	GKASESLQL	263	B5101	17	N.A.
				B2705	17	1
				B2709	16	N.A.
	147-155	ASESLQLVF	264	A1	22	68
	153-161	LVFGIDVKE	265	A26	16	N.A.
				A3	16	<1.0

Table 25B
MAGE-1: Preferred Epitopes Revealed by Housekeeping Proteasome Digestion

Substrate	Epitope	Sequence	Seq. ID No.	Binding Prediction		
				HLA type	SYFPEITHI	NIH
Mage-1 99-125	114-121	LLKYRARE	266	B8	25	<1.0
	106-113	VADLVGFL	267	B8	16	<1.0
				B5101	21	N.A.
				A0201	23	44
				A26	25	N.A.
	105-113	KVADLVGFL	268	A3	16	<5
				B0702	14	20
				B2705	14	30
				A0201	17	<5
	107-115	ADLVGFLL	269	B0702	15	<5
				B2705	16	1
	106-115	VADLVGFLL	270	A0201	16	<5
				A1	22	3
	114-123	LLKYRAREPV	271	A0201	20	2

Table 26
MAGE-3: Preferred Epitopes Revealed by Housekeeping Proteasome Digestion

Substrate	Epitope	Sequence	Seq. ID No.	Binding Prediction		
				HLA type	SYFPEITHI	NIH
Mage-3 267-295	271-278	FLWGPRAL	162	B08	17	<5
	270-278	EFLWGPRAL	163	A26	21	N.A.
				A24	N.A.	30
				B1510	16	N.A.
				A0201	27	2655
	271-279	FLWGPRALV	164	A3	16	2
	278-286	LVETSYVKV	272	A0201	19	<1.0
				A26	17	N.A.
	277-286	ALVETSYVKV	273	A0201	28	428
				A26	16	<5
				A3	18	<5
				A0201	19	27
	285-293	KVLHHMVKI	274	A3	19	<5
	276-284	RALVETSYV	165	A0201	18	20
	283-291	YVKVLHMMV	275	A0201	17	<1.0
	275-283	PRALVETSY	276	A1	17	<1.0
	274-283	GPRALVETSY	277	A1	15	<1.0
	278-287	LVETSYVKVL	278	A0201	18	<1.0
	272-281	LWGPRALVET	168	A0201	16	<1.0
	271-280	FLWGPRALVE	167	A3	22	<5

Table 27A
Fibronectin ED-B: Preferred Epitopes Revealed by Housekeeping Proteasome Digestion

Substrate	Epitope	Sequence	Seq. ID No.	Binding Prediction		
				HLA type	SYFPEITHI	NIH
ED-B 14'-21'	4'-5**	<i>TIIP</i> EV [†] PQL	279	A0201	27	7
				A26	28	N.A.
				A3	17	<5
				B8	15	<5
				B1510	15	N.A.
				B2705	17	10
	5'-5**	<i>DTIIP</i> EV [†] PQL	280	B2709	15	N.A.
				A0201	20	<5
				A26	32	N.A.
	1-10	EVPQLTDLSF	281	A26	29	N.A.

*This substrate contains the 14 amino acids from fibronectin flanking ED-B to the N-terminal side.

**These peptides span the junction between the N-terminus of the ED-B domain and the rest of fibronectin.

† The *italicized* lettering indicates sequence outside the ED-B domain.

Table 27B
Fibronectin ED-B: Preferred Epitopes Revealed by Housekeeping Proteasome Digestion

Substrate	Epitope	Sequence	Seq. ID No.	Binding Prediction		
				HLA type	SYFPEITHI	NIH
ED-B 8-35	23-30	TPSNSSTI	282	B5101	22	N.A.
	18-25	IGLRWTPL	283	B5101	18	N.A.
	17-25	SIGLRWTPL	284	A0201	20	5
				A26	18	N.A.
				B08	25	<5
				A1	19	<5
	25-33	LNSSTIIGY	285	A26	16	<5
	24-33	PLNSSTIIGY	286	A1	20	<5
				A26	24	N.A.
				A3	16	<5
	23-31	TPSNSSTII	287	B0702	17	8
				B5101	25	440

Table 27C
Fibronectin ED-B: Preferred Epitopes Revealed by Housekeeping Proteasome Digestion

Substrate	Epitope	Sequence	Seq. ID No.	Binding Prediction		
				HLA type	SYFPEITHI	NIH
ED-B 20-49	31-38	IGYRITVV	288	B5101	25	N.A.
				A0201	23	15
	30-38	IIGYRITVV	289	A3	17	<1.0
				B08	15	<1.0
				B5101	15	3
				A0201	26	9
	29-38	TIIGYRITVV	290	A26	18	N.A.
				A3	18	<5
	23-30	TPLNSSTI	282	B5101	22	N.A.
	25-33	LNSSTIIGY	285	A1	19	<5
				A26	16	N.A.
	24-33	PLNSSTIIGY	286	A26	24	N.A.
				A3	16	<5
	31-39	IGYRITVVA	291	A3	17	<5
	30-39	IIGYRITVVA	292	A0201	15	<5
				A3	18	<5
23-31	TPLNSSTII		287	B0702	17	8
				B5101	25	440

Table 28A
CEA: Preferred Epitopes Revealed by Housekeeping Proteasome Digestion

Substrate	Epitope	Sequence	Seq. ID No.	Binding Prediction		
				HLA type	SYFPEITHI	NIH
CEA 176-202	184-191	SLPVSPRL	293	B08	19	<5
				A0201	15	<5
	183-191	QSLPVSPRL	294	B1510	15	
				B2705	18	10
				B2709	15	
	186-193	PVSPRLQL	295	B08	18	<5
				B0702	26	180
	185-193	LPVSPRLQL	296	B08	16	<5
				B5101	19	130
	184-193	SLPVSPRLQL	297	A0201	23	21
				A26	18	N.A.
				A3	18	<5
	185-192	LPVSPRLQ	298	B5101	17	N.A.
				A0201	21	4
				A26	16	N.A.
	192-200	QLSNGNRTL	299	A3	19	<5
				B08	17	<5
				B1510	15	
	191-200	LQLSNGNRTL	300	A0201	16	3
	179-187	WVNNQSLPV	301	A0201	16	28
	186-194	PVSPRLQLS	302	A26	17	N.A.
				A3	15	<5

Table 28B CEA: Preferred Epitopes Revealed by Housekeeping Proteasome Digestion						
Substrate	Epitope	Sequence	Seq. ID No.	Binding Prediction		
				HLA type	SYFPEITHI	NIH
CEA 354-380	362-369	SLPVSPRL	303	B08	19	<1.0
	361-369	QSLPVSPRL	304	A0201	15	<1.0
				B2705	18	10
				B2709	15	
	364-371	PVSPRLQL	305	B08	18	<1.0
	363-371	LPVSPRLQL	306	B0702	26	180
				B08	16	<1.0
				B5101	19	130
	362-371	SLPVSPRLQL	307	A0201	23	21
				A26	18	N.A.
				A24	N.A.	6
				A3	18	<5
				B5101	17	N.A.
	363-370	LPVSPRLQ	308	A0201	22	4
	370-378	QLSNDNRTL	309	A26	16	N.A.
				A3	17	<1.0
				B08	17	<1.0
	369-378	LQLSNDNRTL	310	A0201	16	3
	357-365	WVNNQSLPV	311	A0201	16	28
	360-368	NQSLPVSPR	312	B2705	14	100

Table 28C
CEA: Preferred Epitopes Revealed by Housekeeping Proteasome Digestion

Substrate	Epitope	Sequence	Seq. ID No.	Binding Prediction		
				HLA type	SYFPEITHI	NIH
CEA 532-558	540-547	SLPVSPRL	313	B08	19	<5
	539-547	QSLPVSPRL	314	A0201	15	<5
				B1510	15	<5
				B2705	18	10
				B2709	15	
	542-549	PVSPRLQL	315	B08	18	<5
	541-549	LPVSPRLQL	316	B0702	26	180
				B08	16	<1.0
				B5101	19	130
	540-549	SLPVSPRLQL	317	A0201	23	21
				A26	18	N.A.
				A3	18	<5
				B5101	17	N.A.
	541-548	LPVSPRLQ	318	A0201	24	4
	548-556	QLSNGNRTL	319	A26	16	N.A.
				A3	19	<1.0
				B08	17	<1.0
				B1510	15	
	547-556	LQLSNGNRTL	320	A0201	16	3
	535-543	WVNGQSLPV	321	A0201	18	28
				A3	15	<1.0
	533-541	LWWVNGQSL	322	A0201	15	<5

Table 28D
CEA: Preferred Epitopes Revealed by Housekeeping Proteasome Digestion

Substrate	Epitope	Sequence	Seq. ID No.	Binding Prediction		
				HLA type	SYFPEITHI	NIH
CEA 532-558 (continued)	532-541	YLWWVNGQSL	323	A0201	25	816
				A26	18	N.A.
	538-546	GQSLPVSPR	324	B2705	17	100

Table 29A
HER2/NEU: Preferred Epitopes Revealed by Housekeeping Proteasome Digestion

Substrate	Epitope	Sequence	Seq. ID No.	Blinding Prediction		
				HLA type	SYFPEITHI	NIH
Her-2 25-52	30-37	DMKLRIPA	325	B08	19	8
	28-37	GTDMLRIPA	326	A1	23	6
	42-49	HLDMLRHL	327	B08	17	<5
	41-49	THLDMLRHL	328	A0201	17	<5
				B1510	24	N.A.
	40-49	ETHLDMLRHL	329	A26	29	N.A.
	36-43	PASPETHL	330	B5101	17	N.A.
				A0201	15	<5
	35-43	LPASPETHL	331	B5101	20	130
				B5102	N.A.	100
	34-43	RLPASPETHL	332	A0201	20	21
				A0201	15	<5
	38-46	SPETHLDML	333	B0702	20	24
				B08	18	<5
				B5101	18	110
	37-46	ASPETHLDML	334	A0201	18	<5
	42-50	HLDMLRHLY	335	A1	29	25
				A26	20	N.A.
				A3	17	4
	41-50	THLDMLRHLY	336	A1	18	<1.0

Table 29B
HER2/NEU: Preferred Epitopes Revealed by Housekeeping Proteasome Digestion

Substrate	Epitope	Sequence	Seq. ID No.	Binding Prediction		
				HLA type	SYFPEITHI	NIH
Her-2 705-732	719-726	ELRKVKVL	337	B08	24	16
				A0201	16	1
	718-726	TELKVKVL	338	B08	22	<5
				B5101	16	<5
	717-726	ETELRKVKVL	339	A1	18	2
				A26	28	6
	715-723	LKETELRKV	340	A0201	17	<5
				B5101	15	<5
	714-723	ILKETELRKV	341	A0201	29	8
				A0201	15	<5
	712-720	MRILKETEL	342	B08	22	<5
				B2705	27	2000
				B2709	21	N.A.
	711-720	QMRILKETEL	343	A0201	20	2
				B0702	13	40
	717-725	ETELRKVKV	344	A1	18	5
				A26	18	N.A.
	716-725	KETELRKVKV	345	A0201	16	19
	706-714	MPNQAQMRI	346	B0702	16	8
				B5101	22	629
	705-714	AMPNQAQMRI	347	A0201	18	8
	706-715	MPNQAQMRL	348	B0702	20	80

Table 29C
HER2/NEU: Preferred Epitopes Revealed by Housekeeping Proteasome Digestion

Substrate	Epitope	Sequence	Seq. ID No.	Binding Prediction		
				HLA type	SYFPEITHI	NIH
Her-2 954-982	966-973	RPRFREL V	349	B08	20	24
				B5101	18	N.A.
	965-973	CRPRFREL V	350	B2709	18	
				A26	25	N.A.
				A24	N.A.	32
	968-976	RFREL VSEF	351	A3	15	<5
				B08	16	<5
				B2705	19	
	967-976	PRFREL VSEF	352	A26	18	N.A.
				A26	21	N.A.
				A24	N.A.	6
	964-972	ECRPRREL	353	B0702	15	40
				B8	27	640
				B1510	16	<5

Table 30
 NY-ESO-1: Preferred Epitopes Revealed by Housekeeping Proteasome Digestion

Substrate	Epitope	Sequence	Seq. ID No.	Binding Prediction		
				HLA type	SYFPEITHI	NIH
NY-ESO-1 51-77	67-75	GAASGLNGC	354	A0201	15	<5
	52-60	RASGPGGGA	355	B0702	15	<5
	64-72	PHGGAASGL	356	B1510	21	N.A.
	63-72	GPHGGAASGL	357	B0702	22	80
	60-69	APRGPHGGAA	358	B0702	23	60

Table 31A
PRAME: Preferred Epitopes Revealed by Housekeeping Proteasome Digestion

Substrate	Epitope	Sequence	Seq. ID No.	Binding Prediction		
				HLA type	SYFPEITHI	NIH
PRAME 103-135	112-119	VRPRRWKL	359	B08	19	
	111-119	EVRPRRWKL	360	A26	27	N.A.
				A24	N.A.	5
				A3	19	N.A.
				B0702	15	(B7) 300.00
	113-121	RPRRWKLQV	361	B08	26	160
				B0702	21	(B7) 40.00
				B5101	19	110
	114-122	PRRWKLQVL	362	B08	26	<5
				B2705	23	200
	113-122	RPRRWKLQVL	363	B0702	24	(B7) 800.00
				B8	N.A.	160
				B5101	N.A.	61
				B5102	N.A.	61
PRAME 161-187	116-124	RWKLQVLDL	364	A24	N.A.	10
				B08	22	<5
	115-124	RRWKLQVLDL	365	B2705	17	3
	174-182	PVEVLVDLF	366	A0201	16	<5
				A26	25	N.A.

Table 31B
PRAME: Preferred Epitopes Revealed by Housekeeping Proteasome Digestion

Substrate	Epitope	Sequence	Seq. ID No.	Binding Prediction		
				HLA type	SYRPEITHI	NIH
PRAME 185-215	199-206	VKRKKKNVL	367	B08	27	8
				A0201	16	<1.0
				A26	20	N.A.
	198-206	KVKRKKKNVL	368	A3	22	<1.0
				B08	30	40
				B2705	16	
	197-206	EKVKRKKKNVL	369	A26	15	N.A.
	198-205	KVKRKKKNV	370	B08	20	6
	201-208	RKKKNVLR	371	B08	20	<5
				A0201	15	<1.0
				A26	15	N.A.
	200-208	KRKKNVLR	372	B0702	15	<1.0
				B08	21	<1.0
				B2705	28	
				B2709	25	
	199-208	VKRKKKNVLR	373	A0201	16	<1.0
				B0702	16	4
	189-196	DELFSYLI	374	B5101	15	N.A.
				A0201	22	3
	205-213	VLRLOCKKL	375	A26	17	N.A.
				B08	25	8
	204-213	NVLRLOCKKL	376	A0201	17	7
				A26	19	N.A.

Substrate	Epitope	Sequence	Seq. ID No.	Binding Prediction		
				HLA type	SYRPEITHI	NIH
PRAME 185-215 (continued)	194-202	YLIEKVKRK	377	A0201	20	<1.0
				A26	18	N.A.
				A3	25	68
				B08	20	<1.0
				B2705	17	
PRAME 71-98	74-81	QAWPFTCL	378	B5101	17	n.a.
	73-81	VQAWPFTCL	379	A0201	14	7
				A24	n.a.	5
				B0702	16	6
	72-81	MVQAWPFTCL	380	A26	22	n.a.
				A24	n.a.	7
				B0702	13	30
	81-88	LPLGVLMK	381	B5101	18	n.a.
	80-88	CLPLGVLMK	382	A0201	17	<1.0
				A3	27	120
	79-88	TCLPLGVLMK	383	A1	12	10
				A3	19	3
				A0201	18	7
	84-92	GVLKGGQHL	384	A26	21	n.a.
	81-89	LPLGVLMKG	385	B08	21	4
				B5101	20	2
				A0201	16	<1.0
	76-85	WPFTCLPLGV	387	B0702	18	4

Table 31D
PRAME: Preferred Epitopes Revealed by Housekeeping Proteasome Digestion

Substrate	Epitope	Sequence	Seq. ID No.	Binding Prediction		
				HLA type	SYFPEITHI	NIH
PRAME 39-65	51-59	ELFPPLFMA	388	A0201	19	18
				A26	23	N.A.
	49-57	PRELFPPLF	389	B2705	22	
				B2709	19	
	48-57	LPRELFPPLF	390	B0702	19	4
				B2705	16	
PRAME 39-65	50-58	RELFPPLFM	391	B2705	15	
	49-58	PRELFPPLFM	392	A1	16	<1.0

Table 32
PSA: Preferred Epitopes Revealed by Housekeeping Proteasome Digestion

Substrate	Epitope	Sequence	Seq. ID No.	Binding Prediction		
				HLA type	SYFPEITHI	NIH
PSA 232-258	239-246	RPSLYTKV	393	B5101	21	N.A.
	238-246	ERPSLYTKV	394	B2705	15	60
	236-243	LPERPSLY	395	B5101	18	N.A.
				A1	19	<1.0
				A26	22	N.A.
				A3	26	6
				B08	16	<1.0
				B2705	11	15
				B2709	19	N.A.
				A0201	20	<1.0
PSA 232-258				A1	19	<1.0
				A26	25	N.A.
				A3	26	60
				B08	20	<1.0
				B2705	13	75
				A1	20	<1.0
				A26	16	N.A.
				B0702	21	4
				B5101	23	110

Table 33A
PSMA: Preferred Epitopes Revealed by Housekeeping Proteasome Digestion

Substrate	Epitope	Sequence	Seq. ID No.	Binding Prediction		
				HLA type	SYFPEITHI	NIH
PSMA 202-228	211-218	GNKVKNAQ	400	B08	22	<5
	202-209	IARYGKVF	401	B08	18	<5
	217-225	AQLAGAKGV	402	A0201	16	26
	207-215	KVFRGNKVK	403	A3	32	15
	211-219	GNKVKNAQL	404	B8	33	80
PSMA 255-282	269-277	TPGYPANEY	405	B2705	17	20
	268-277	LTPGYPANEY	406	A1	16	<5
	271-279	GYPANEYAY	407	A1	21	1
	270-279	PGYPANEYAY	408	A26	24	N.A.
	266-274	DPLTPGYPA	409	A1	15	<5
PSMA 483-509	492-500	SLYESWTKK	410	A1	19	<5
	491-500	KSLYESWTKK	411	B0702	21	3
	486-494	EGFEGKSLY	412	B5101	17	20
	485-494	DEGFEGKSLY	413	A0201	17	<5
	498-506	TKKSPSPEF	414	A3	27	150
				B2705	18	150
				A3	16	<5
				A1	19	<5
				A26	21	N.A.
				B2705	16	<5
				A1	17	<5
				A26	17	N.A.
				B08	17	<5

Table 33B
PSMA: Preferred Epitopes Revealed by Housekeeping Proteasome Digestion

Substrate	Epitope	Sequence	Seq. ID No.	Binding Prediction		
				HLA type	SYFPEITHI	NIH
PSMA 483-509 (continued)	497-506	WTKKSPSEF	415	A26	24	N.A.
	492-501	SLYESWTKKS	416	A0201	16	<5
				A3	16	<5
	725-732	WGEVKRQI	417	B08	17	<5
				B5101	17	N.A.
	724-732	AWGEVKRQI	418	B5101	15	6
	723-732	KAWGEVKRQI	419	A0201	16	<1.0
	723-730	KAWGEVKR	420	B5101	15	N.A.
	722-730	SKAWGEVKR	421	B2705	15	<5
				A0201	21	177
	731-739	QIYVAAFTV	422	A3	21	<1.0
				B5101	15	5
PSMA 721-749				A0201	17	6
	733-741	YVAAFTVQA	423	A3	20	<1.0
	725-733	WGEVKRQIY	424	A1	26	11
	727-735	EVKRQIYVA	425	A26	22	N.A.
				A3	18	<1.0
	738-746	TVQAAAETL	426	A26	18	N.A.
				A3	19	<1.0
	737-746	FTVQAAAETL	427	A0201	17	<1.0
				A26	19	N.A.

Table 33C
PSMA: Preferred Epitopes Revealed by Housekeeping Proteasome Digestion

Substrate	Epitope	Sequence	Seq. ID No.	Binding Prediction		
				HLA type	SYFPEITHI	NIH
PSMA 721-749 (continued)	729-737	KRQYVAAF	428	A26	16	N.A.
				B2705	24	3000
				B2709	21	N.A.
	721-729	PSKAWGEVK	429	A3	20	<1.0
PSMA 95-122	723-731	KAWGEVKRQ	430	B5101	16	<1.0
	100-108	WKEFGLDV	431	A0201	16	<5
	99-108	QWKEFGLDV	432	A0201	17	<5
	102-111	EFGLDSVELA	433	A26	16	N.A.

Table 34A
SCP-1: Preferred Epitopes Revealed by Housekeeping Proteasome Digestion

Substrate	Epitope	Sequence	Seq. ID No.	Binding Prediction		
				HLA type	SYRPEITHI	NIH
SCP-1 117-143	126-134	ELRQESKL	434	A0201	20	<5
				A26	26	N.A.
				A3	17	<5
				B0702	13	(B7) 40.00
				B8	34	320
SCP-1 281-308	125-134	AE LRQESKL	435	A0201	16	<5
	133-141	KLQENRKII	436	A0201	20	61
	298-305	QLEEKTKL	437	B08	28	2
	297-305	NQLEEKTKL	438	A0201	16	33
				B2705	19	200
	288-296	LLEESRD KV	439	A0201	25	15
				B5101	15	3
				A0201	27	2378
	287-296	FLLEESRD KV	440	A26	21	N.A.
	291-299	ESRD KV NQL	441	B08	29	240
SCP-1 471-498	290-299	EE SRD KV NQL	442	A26	19	N.A.
	475-483	EKEVHDLEY	443	A1	31	11
	474-483	REKEVHDLEY	444	A26	17	N.A.
				A1	21	<1.0
	480-488	DLEYSYCHY	445	A1	26	45
				A26	30	N.A.
	477-485	EVHDLEYSY	446	A3	16	<5
				A1	15	1

Table 34B

SCP-1: Preferred Epitopes Revealed by Housekeeping Proteasome Digestion

Substrate	Epitope	Sequence	Seq. ID No.	Binding Prediction		
				HLA type	SYFPEITHI	NIH
SCP-1 471-498 (continued)	477-485	EVHDLSEYSY		A26	29	N.A.
	477-486	EVHDLSEYSYC	447	A3	19	<1.0
	502-509	KLSSKREL	448	A26	22	N.A.
	508-515	ELKNTSEYF	449	B08	26	4
	507-515	RELKNTSEYF	450	B08	24	<1.0
	496-503	KRGQRPKL	451	B2705	18	45
SCP-1 493-520				B4403	N.A.	120
				B08	18	<1.0
				B0702	22	120
	494-503	LPKRGQRPKL	452	B8	N.A.	16
				B5101	N.A.	130
				B3501	N.A.	60
	509-517	LKNTSEYFTL	453	A0201	15	<5
				A0201	18	<1.0
	508-517	ELKNTSEYFTL	454	A26	27	N.A.
				A3	16	<1.0
	506-514	KRELKNTSEY	455	A1	26	2
				B2705	26	3000
	502-510	KLSSKRELK	456	A3	25	60
	498-506	GQRPKLSSK	457	A3	22	4
	497-506	RQRPKLSSK	458	B2705	18	200
	500-508	RPKLSSKRE	459	A3	22	<1.0
				B08	18	<1.0

Table 34C
SCP-1: Preferred Epitopes Revealed by Housekeeping Proteasome Digestion

Substrate	Epitope	Sequence	Seq. ID No.	Binding Prediction		
				HLA type	SYFPEITHI	NIH
SCP-1 570-596	573-580	LEYVREEL	460	B08	19	<5
				A0201	17	<1.0
	572-580	ELEYVREEL	461	A26	23	N.A.
				A24	N.A.	9
				B08	20	N.A.
	571-580	N ELEYVREEL	462	A0201	16	4
				A0201	19	<1.0
	579-587	ELKQKRDEV	463	A26	18	N.A.
				B08	29	48
	575-583	YVREELKQK	464	A26	17	N.A.
SCP-1 618-645				A3	27	2
	632-640	QLNVYEIKV	465	A0201	24	70
	630-638	SKQLNVYEI	466	A0201	17	<5
	628-636	AESKQLNVY	467	A1	19	<5
				A26	16	N.A.
	627-636	TAESKQLNVY	468	A1	26	45
				A26	15	N.A.

Table 34D
SCP-1: Preferred Epitopes Revealed by Houskeeping Proteasome Digestion

Substrate	Epitope	Sequence	Seq. ID No.	Binding Prediction		
				HLA type	SYFPEITHI	NIH
SCP-1 633-660	638-645	IKV NKLEL	469	B08	21	<1.0
				A0201	17	<1.0
	637-645	EIKV NKLEL	470	A26	26	N.A.
				B08	28	8
				B1510	15	N.A.
	636-645	YEIKV NKLEL	471	A0201	17	2
	642-650	KLELEESA	472	A0201	20	1
				A3	16	<1.0
	635-643	VYEIKV NKL	473	A0201	18	<1.0
				A24	N.A.	396
SCP-1 640-668				B08	22	<1.0
	634-643	NVYEIKV NKL	474	A0201	24	56
				A26	25	N.A.
				A24	N.A.	6
				A3	15	<5
				B0702	11	(B7) 20
	646-654	ELESAKQKF	475	B08	N.A.	6
	642-650	KLELEESA	476	A26	27	N.A.
				A0201	20	1
	646-654	ELESAKQKF	477	A3	16	<1.0
				A26	27	N.A.

Table 34E
SCP-1: Preferred Epitopes Revealed by Housekeeping Proteasome Digestion

Substrate	Epitope	Sequence	Seq. ID No.	Binding Prediction		
				HLA type	SYFPEITHI	NIH
SCP-1 768-796	771-778	KEKLKREA	478	B08	21	<5
				A0201	18	<5
				A26	18	N.A.
				A24	N.A.	5
	777-785	EAKENTATL	479	B0702	13	12
				B08	28	48
				B5101	20	121
	776-785	REAKENTATL	480	A0201	16	<5
	773-782	KLKREAKENT	481	A3	17	<5
	112-119	EAEKIKKW	482	B5101	17	N.A.
SCP-1 92-125				A0201	23	32
				A26	22	N.A.
	101-109	GLSRVYSKL	483	A24	N.A.	6
				A3	17	3
				B08	17	<1.0
	100-109	EGLSRVYSKL	484	A26	21	N.A.
				A24	N.A.	9
	108-116	KLYKEAEKI	485	A0201	22	57
				A3	20	9
				B5101	18	5
	98-106	NSEGLSRVY	486	A1	31	68
	97-106	ENSEGLSRVY	487	A26	18	N.A.
	102-110	LSRVYSKLY	488	A1	22	<1.0

Table 34F SCP-1: Preferred Epitopes Revealed by Housekeeping Proteasome Digestion					Binding Prediction	
Substrate	Epitope	Sequence	Seq. ID No.	HLA type	SYFPEITHI	NIH
SCP-1 92-125 (continued)	101-110	GLSRVYSKLY	489	A1	18	<1.0
				A26	18	N.A.
				A3	19	18
	96-105	LENSEGLSRV	490	A0201	17	5
SCP-1 931-958	108-117	KLYKEAEKJK	491	A3	27	150
	949-956	REDRWAVI	492	B5101	15	N.A.
				B2705	18	600
	948-956	MREDRWAVI	493	B2709	18	N.A.
				B5101	15	1
				A0201	21	6
	947-956	KMREDRWAVI	494	B08	N.A.	15
	947-955	KMREDRWAV	495	A0201	22	411
	934-942	TTPGSTLKF	496	A26	25	N.A.
	933-942	LTPGSTLKF	497	A26	23	N.A.
SCP-1 232-259	937-945	GSTLKFAGI	498	B08	19	1
	945-953	IRKMREDRW	499	B08	19	<5
	236-243	RLEMHFKL	500	B08	16	<5
				A0201	18	<5
	235-243	SRLEMHFKL	501	B2705	25	2000
				B2709	22	
	242-250	KLKEDYEKI	502	A0201	22	4

Table 34G
SCP-1: Preferred Epitopes Revealed by Housekeeping Proteasome Digestion

Substrate	Epitope	Sequence	Seq. ID No.	Binding Prediction		
				HLA type	SYFPEITHI	NIH
SCP-1 232-259 (continued)				A26	16	N.A.
				A3	15	3
				B08	24	<5
				B5101	14	2
				A1	15	<5
	249-257	KIQHLEQY	503	A26	23	N.A.
				A3	17	<5
				A1	15	<5
	248-257	EKIQHLEQY	504	A26	21	N.A.
	233-242	ENSRLEMHF	505	A26	19	N.A.
SCP-1 310-340	236-245	RLEMHFKLKE	506	A1	19	<5
				A3	17	<5
	324-331	LEDIKVSL	507	B08	20	<1.0
				A0201	21	<1.0
				A26	25	N.A.
	323-331	ELEDIKVSL	508	A24	N.A.	10
				A3	17	<1.0
				B08	19	<1.0
				B1510	16	N.A.
	322-331	KELEDIKVSL	509	A0201	19	22
	320-327	LTKLEDI	500	B08	18	<5
	319-327	HLTKLEDI	511	A0201	21	<1.0
	330-338	SLQRSVSTQ	512	A0201	18	<1.0

Table 34H
SCP-1: Preferred Epitopes Revealed by Housekeeping Proteasome Digestion

Substrate	Epitope	Sequence	Seq. ID No.	Binding Prediction		
				HLA type	SYFPEITHI	NIH
SCP-1 310-340 (continued)	321-329	TKELEDKV	513	A1	16	<1.0
	320-329	LTKLEDKV	514	A0201	19	<1.0
	326-335	DIKVSLSQSV	515	A26	18	N.A.
	281-288	KMKDLTFL	516	B08	20	3
	280-288	NKMKDLTFL	517	A0201	15	1
SCP-1 272-305	279-288	ENKMKDLTFL	518	A26	19	N.A.
	288-296	LLEESRDKV	519	A0201	25	15
	287-296	FLLEESRDKV	520	B5101	15	3
	291-299	ESRDKNVQL	521	A0201	27	2378
	290-299	EESRDKNVQL	522	A26	21	N.A.
	277-285	EKENKMKDL	523	B08	29	240
	276-285	TEKENKMKDL	524	A26	19	N.A.
	279-287	ENKMKDLTF	525	A26	19	N.A.
	218-225	IEKMITAF	526	B08	28	4
	217-225	NIEKMITAF	527	B08	17	<5
SCP-1 211-239	216-225	SNIEKMITAF	528	A26	26	N.A.
	223-230	TAFEELRV	529	A26	19	N.A.
	222-230	ITAFEELRV	530	B5101	23	N.A.
	221-230	MITAFEELRV	531	A0201	18	2
				A0201	18	16

Table 341
SCP-1: Preferred Epitopes Revealed by Housekeeping Proteasome Digestion

Substrate	Epitope	Sequence	Seq. ID No.	Binding Prediction		
				HLA type	SYFPEITHI	NIH
SCP-1 211-239 (continued)	220-228	KMITAFEEL	532	A0201	23	50
				A26	15	N.A.
				A24	N.A.	16
	219-228	EKMITAFEEL	533	A26	19	N.A.
				A3	16	<1.0
	227-235	ELRVQAENS	534	B08	15	<1.0
				A0201	17	<1.0
	213-222	DLNSNIEKMI	535	A26	16	N.A.
				B08	20	4
	837-844	WTSAKNTL	536	A0201	18	2
SCP-1 836-863	846-854	TPLPKAYTV	537	B0702	17	4
				B08	16	2
				B5101	25	220
	845-854	STPLPKAYTV	538	A0201	19	<5
	844-852	LSTPLPKAY	539	A1	23	8
				A1	16	<1.0
	843-852	TLSTPLPKAY	540	A26	19	N.A.
				A3	18	2
	842-850	NTLSTPLPK	541	A3	16	3
	841-850	KNTLSTPLPK	542	A3	18	<1.0

Table 34J
SCP-1: Preferred Epitopes Revealed by Housekeeping Proteasome Digestion

Substrate	Epitope	Sequence	Seq. ID No.	Blinding Prediction		
				HLA type	SYFPEITHI	NIH
SCP-1 819-845	828-835	ISKDKRDY	543	B08	21	3
				A26	21	N.A.
	826-835	HGSKDKRDY	544	A1	15	<5
	832-840	KRDYLTSA	545	B2705	16	600
	829-838	SKDKRDYLT	546	A1	18	<5
SCP-1 260-288	279-286	ENKMKDLT	547	B08	22	8
				A0201	17	3
	260-268	EINDKEKQV	548	A26	19	N.A.
				B08	17	<5
	274-282	QTTEKENKM	549	A0201	17	3
				A26	22	N.A.
	269-277	SLLLIQITE	550	B08	16	<5
				A0201	16	<1.0
				A3	18	<1.0
	453-460	FEKIAEEL	551	B08	21	<1.0
SCP-1 437-464	452-460	QFEKIAEEL	552	B2705	15	
	451-460	KQFEKIAEEL	553	A0201	16	56
	449-456	DNKQFEKI	554	B08	16	2
				B5101	16	N.A.
	448-456	YDNKQFEKI	555	B5101	16	1
	447-456	LYDNKQFEKI	556	A1	15	<1.0

Table 34K
SCP-1: Preferred Epitopes Revealed by Housekeeping Proteasome Digestion

Substrate	Epitope	Sequence	Seq. ID No.	Blinding Prediction		
				HLA type	SYFPEITHI	NIH
SCP-1 437-464 (continued)	440-447	LGEKETLL	557	B5101	16	N.A.
	439-447	VLGEKETLL	558	A0201	24	149
				A26	19	N.A.
				B08	29	12
	438-447	KVLGEKETLL	559	A0201	19	24
				A26	20	N.A.
				A24	N.A.	12
				A3	18	<1.0
				B0702	14	20
				A0201	22	3
SCP-1 383-412	390-398	LLRTEQQRL	560	A26	18	N.A.
				B08	22	1.6
				B2705	15	30
				A0201	19	6
	389-398	ELLRTEQQRL	561	A26	24	N.A.
				A3	15	<1.0
				A1	15	<5
	393-401	TEQQRLNY	562	A26	16	N.A.
	392-401	RTEQQRLNY	563	A1	31	113
				A26	26	N.A.
	402-410	EDQLILTM	564	A26	18	N.A.
	397-406	RLENYEDQLI	565	A0201	17	<1.0
				A3	15	<1.0

Table 34K
SCP-1: Preferred Epitopes Revealed by Housekeeping Proteasome Digestion

Substrate	Epitope	Sequence	Seq. ID No.	Binding Prediction		
				HLA type	SYFPEITHI	NIH
SCP-1 366-394	368-375	KARAAHSF	566	B08	16	<1.0
	376-384	VVTEFETTV	567	A0201	19	161
	375-384	FVVTEFETTV	568	A3	16	<1.0
	377-385	VTEFETIVC	569	A0201	17	106
	376-385	VVTEFETIVC	570	A1	18	2
SCP-1 331-357				A3	16	<5
	344-352	DLQIATNTI	571	A0201	22	<5
				A3	15	<1.0
				B5101	17	11
	347-355	IATNTICQL	572	A0201	19	1
				B08	16	<1.0
	346-355	QIATNTICQL	573	B5101	20	79
				A0201	24	7
				A26	24	N.A.

Table 35
 SSX-4: Preferred Epitopes Revealed by Housekeeping Proteasome Digestion

Substrate	Epitope	Sequence	Seq. ID No.	Binding Prediction		
				HLA type	SYFPEITHI	NIH
SSX4 45-76	57-65	VMTKLGFKV	574	A0201	21	495
	53-61	LNVEVMTKL	575	A0201	17	7
	52-61	KLNYEVMTKL	576	A0201	23	172
				A26	21	N.A.
				A24	N.A.	18
				A3	14	4
				B7	N.A.	4
SSX4 98-124	66-74	TLPPFMRSK	577	A26	16	N.A.
	110-118	KMPKKPAE	578	A3	25	14
				A0201	15	<5
SSX4 98-124	103-112	SLQRFPKIM	579	A26	15	N.A.
				A3	16	<5
				A0201	15	8
				A26	16	N.A.

Table 36 Tyrosinase: Preferred Epitopes Revealed by Housekeeping Proteasome Digestion						
Substrate	Epitope	Sequence	Seq. ID No.	Binding Prediction		
				HLA type	SYFPEITHI	NIH
Tyr 445-474	463-471	YKSYLEQA	580	A0201	18	<5
				A26	17	N.A.
	459-467	SFQDYKSY	581	A1	18	<5
				A26	22	N.A.
	458-467	DSFQDYIKSY	582	A1	19	<5
Tyr 490-518				A26	24	N.A.
	507-514	LPEEKQPL	583	B08	28	5
				B5101	18	N.A.
				A0201	22	88
	506-514	QLPEEKQPL	584	A26	20	N.A.
				A24	N.A.	9
				B08	18	<5
	505-514	KQLPEEKQPL	585	A0201	15	28
				A24	N.A.	17
	507-515	LPEEKQPLL	586	A0201	15	<5
Tyr 490-518				B0702	21	24
				B08	28	5
				B5101	21	157
	506-515	QLPEEKQPLL	587	A0201	23	88
				A26	20	N.A.
497-505	SLICRHKRK	588	A24	N.A.	7	
			A3	25	15	

Example 15**Evaluating Likelihood of Epitope Cross-reactivity on Non-target Tissues.**

As noted above PSA is a member of the kallikrein family of proteases, which is itself a subset of the serine protease family. While the members of this family sharing the greatest degree of sequence identity with PSA also share similar expression profiles, it remains possible that individual epitope sequences might be shared with proteins having distinctly different expression profiles. A first step in evaluating the likelihood of undesirable cross-reactivity is the identification of shared sequences. One way to accomplish this is to conduct a BLAST search of an epitope sequence against the SWISSPROT or Entrez non-redundant peptide sequence databases using the "Search for short nearly exact matches" option; hypertext transfer protocol accessible on the world wide web (<http://www.ncbi.nlm.nih.gov/blast/index.html>). Thus searching SEQ ID NO. 214, WVLTAAHCL, against SWISSPROT (limited to entries for homo sapiens) one finds four exact matches, including PSA. The other three are from kallikrein 1 (tissue kallikrein), and elastase 2A and 2B. While these nine amino acid segments are identical, the flanking sequences are quite distinct, particularly on the C-terminal side, suggesting that processing may proceed differently and that thus the same epitope may not be liberated from these other proteins. (Please note that kallikrein naming is confused. Thus the kallikrein 1 [accession number P06870] is a different protein than the one [accession number AAD13817] mentioned in the paragraph on PSA above in the section on tumor-associated antigens).

It is possible to test this possibility in several ways. Synthetic peptides containing the epitope sequence embedded in the context of each of these proteins can be subjected to *in vitro* proteasomal digestion and analysis as described above. Alternatively, cells expressing these other proteins, whether by natural or recombinant expression, can be used as targets in a cytotoxicity (or similar) assay using CD8⁺ T cells that recognize the epitope, in order to determine if the epitope is processed and presented.

Example 16**Epitope Clusters.**

Known and predicted epitopes are generally not evenly distributed across the sequences of protein antigens. As referred to above, we have defined segments of sequence containing a higher than average density of (known or predicted) epitopes as epitope clusters. Among the uses of epitope clusters is the incorporation of their sequence into substrate peptides used in proteasomal digestion analysis as described herein. Epitope clusters can also be useful as vaccine components. A fuller discussion of the definition and uses of epitope clusters is found in U.S. Patent Application No. 09/561,571 entitled EPITOPE CLUSTERS, previously incorporated by reference.

The following tables (37-60) present 9-mer epitopes predicted for HLA-A2 binding using both the SYFPEITHI and NIH algorithms and the epitope density of regions of overlapping

epitopes, and of epitopes in the whole protein, and the ratio of these two densities. (The ratio must exceed one for there to be a cluster by the above definition; requiring higher values of this ratio reflect preferred embodiments). Individual 9-mers are ranked by score and identified by the position of their first amino in the complete protein sequence. Each potential cluster from a protein is numbered. The range of amino acid positions within the complete sequence that the cluster covers is indicated as are the rankings of the individual predicted epitopes it is made up of.

Table 37
BIMAS-NIH/Parker algorithm Results for gp100

Rank	Start	Score	Rank	Start	Score
1	619	1493	21	416	19
2	602	413	22	25	18
3	162	226	23	566	17
4	18	118	24	603	15
5	178	118	25	384	14
6	273	117	26	13	14
7	601	81	27	290	12
8	243	63	28	637	10
9	606	60	29	639	9
10	373	50	30	485	9
11	544	36	31	453	8
12	291	29	32	102	8
13	592	29	33	399	8
14	268	29	34	456	7
15	47	27	35	113	7
16	585	26	36	622	7
17	576	21	37	69	7
18	465	21	38	604	6
19	570	20	39	350	6
20	9	19	40	583	5

Table 38
SYFPEITHI (Rammensee algorithm) Results for gp100

Rank	Start	Score	Rank	Start	Score	Rank	Start	Score
1	606	30	37	291	20	73	60	18
2	162	29	38	269	20	74	17	18
3	456	28	39	2	20	75	613	17
4	18	28	40	610	19	76	599	17
5	602	27	41	594	19	77	572	17
6	598	27	42	591	19	78	557	17
7	601	26	43	583	19	79	556	17
8	597	26	44	570	19	80	512	17
9	13	26	45	488	19	81	406	17
10	585	25	46	446	19	82	324	17
11	449	25	47	322	19	83	290	17
12	4	25	48	267	19	84	101	17
13	603	24	49	250	19	85	95	17
14	576	24	50	205	19	86	635	16
15	453	24	51	180	19	87	588	16
16	178	24	52	169	19	88	584	16
17	171	24	53	88	19	89	577	16
18	11	24	54	47	19	90	559	16
19	619	23	55	10	19	91	539	16
20	280	23	56	648	18	92	494	16
21	268	23	57	605	18	93	482	16
22	592	22	58	604	18	94	468	16
23	544	22	59	595	18	95	442	16
24	465	22	60	571	18	96	413	16
25	399	22	61	569	18	97	408	16
26	373	22	62	450	18	98	402	16
27	273	22	63	409	18	99	286	16
28	243	22	64	400	18	100	234	16
29	566	21	65	371	18	101	217	16
30	563	21	66	343	18	102	211	16
31	485	21	67	298	18	103	176	16
32	384	21	68	209	18	104	107	16
33	350	21	69	102	18	105	96	16
34	9	21	70	97	18	106	80	16
35	463	20	71	76	18	107	16	16
36	397	20	72	69	18	108	14	16
						109	7	16

Table 39

Prediction of clusters for gp100

Total AAs: 661

Total 9-mers: 653

SYFPEITHI 16: 109 9-mers

NIH 5: 40 9-mers

	Cluster #	AAs	Epitopes (by Rank)	Epitopes/AA		
				Cluster	Whole Pr	Ratio
SYFPEITHI	1	2 to 26	39, 12, 109, 34, 55, 11, 9, 108, 107, 74, 4	0.440	0.165	2.668
	2	69-115	72, 71, 106, 53, 85, 105, 70, 84, 69, 104	0.213	0.165	1.290
	3	95-115	85, 105, 70, 84, 69	0.238	0.165	1.444
	4	162-188	2, 52, 17, 103, 16, 51	0.222	0.165	1.348
	5	205-225	50, 68, 102, 101	0.190	0.165	1.155
	6	243-258	28, 49	0.125	0.165	0.758
	7	267-306	48, 21, 38, 27, 20, 99, 83, 37, 67	0.225	0.165	1.364
	8	322-332	47, 82	0.182	0.165	1.103
	9	343-358	66, 33	0.125	0.165	0.758
	10	371-381	65, 26	0.182	0.165	1.103
	11	397-421	36, 25, 64, 98, 81, 97, 63, 96	0.320	0.165	1.941
	12	442-476	95, 46, 11, 62, 15, 3, 35, 24, 94	0.257	0.165	1.559
	13	482-502	93, 31, 45, 93	0.190	0.165	1.155
	14	539-552	91, 23	0.143	0.165	0.866
	15	556-627	79, 78, 90, 30, 29, 61, 44, 60, 77, 14, 89, 43, 88, 10, 87, 42, 22, 41, 59, 8, 6, 76, 7, 5, 13, 58, 57, 1, 40, 75, 19	0.431	0.165	2.611
NIH	1	9 to 33	20, 26, 4, 22	0.160	0.061	2.644
	2	268-281	14, 6	0.143	0.061	2.361
	3	290-299	27, 12	0.200	0.061	3.305
	4*	102-121	32, 35	0.100	0.061	1.653
	5*	373-392	10, 25	0.100	0.061	1.653
	6	453-473	31, 34, 18	0.143	0.061	2.361
	7	566-600	23, 19, 17, 40, 16, 13	0.171	0.061	2.833
	8	601-614	7, 2, 24, 38, 9	0.357	0.061	5.902
	9	619-630	1, 36	0.17	0.061	2.754
	10	637-647	28, 29	0.18	0.061	3.005

*Nearby but not overlapping epitopes

Table 40
BIMAS-NIH/Parker algorithm Results for PSMA

Rank	Start	Score
1	663	1360
2	711	1055
3	4	485
4	27	400
5	26	375
6	668	261
7	707	251
8	469	193
9	731	177
10	35	67
11	33	64
12	554	59
13	427	50
14	115	47
15	20	40
16	217	26
17	583	24
18	415	19
19	193	14
20	240	12
21	627	11
22	260	10
23	130	10
24	741	9
25	3	9
26	733	8
27	726	7
28	286	6
29	174	5
30	700	5

Table 41
SYFPEITHI (Rammensee algorithm) Results for PSMA

Rank	Start	Score	Rank	Start	Score	Rank	Start	Score
1	469	27	31	26	20	61	305	17
2	27	27	32	3	20	62	304	17
3	741	26	33	583	19	63	286	17
4	711	26	34	579	19	64	282	17
5	354	25	35	554	19	65	169	17
6	4	25	36	550	19	66	142	17
7	663	24	37	547	19	67	122	17
8	130	24	38	390	19	68	738	16
9	57	24	39	219	19	69	634	16
10	707	23	40	193	19	70	631	16
11	260	23	41	700	18	71	515	16
12	20	23	42	472	18	72	456	16
13	603	22	43	364	18	73	440	16
14	218	22	44	317	18	74	385	16
15	109	22	45	253	18	75	373	16
16	731	21	46	91	18	76	365	16
17	668	21	47	61	18	77	361	16
18	660	21	48	13	18	78	289	16
19	507	21	49	733	17	79	278	16
20	454	21	50	673	17	80	258	16
21	427	21	51	671	17	81	247	16
22	358	21	52	642	17	82	217	16
23	284	21	53	571	17	83	107	16
24	115	21	54	492	17	84	100	16
25	33	21	55	442	17	85	75	16
26	606	20	56	441	17	86	37	16
27	568	20	57	397	17	87	30	16
28	473	20	58	391	17	88	21	16
29	461	20	59	357	17			
30	200	20	60	344	17			

Table 42

Prediction of clusters for prostate-specific membrane antigen (PSMA)

Total AAs: 750

Total 9-mers: 742

SYFPEITHI 16: 88 9-mers

NIH 5: 30 9-mers

	Cluster #	Aas	Epitopes (by rank)	Epitopes/AA		
				Cluster	Whole Pr	Ratio
SYFPEITHI	1	3 to 12	32, 6	0.200	0.117	1.705
	2	13-45	13, 12, 88, 31, 2, 87, 25, 86	0.242	0.117	2.066
	3	57-69	9, 47	0.154	0.117	1.311
	4	100-138	84, 83, 15, 24, 67, 8	0.154	0.117	1.311
	5	193-208	40, 30	0.111	0.117	0.947
	6	217-227	82, 14, 39	0.273	0.117	2.324
	7	247-268	81, 45, 80, 11	0.182	0.117	1.550
	8	278-297	79, 64, 23, 63, 78	0.250	0.117	2.131
	9	354-381	5, 59, 22, 77, 43, 76, 75	0.250	0.117	2.131
	10	385-405	74, 38, 58, 57	0.190	0.117	1.623
	11	440-450	73, 56, 55	0.273	0.117	2.324
	12	454-481	20, 72, 29, 1, 42, 28	0.214	0.117	1.826
	13	507-523	17, 71	0.118	0.117	1.003
	14	547-562	37, 36, 35	0.188	0.117	1.598
	15	568-591	27, 53, 34, 33	0.167	0.117	1.420
	16	603-614	13, 26	0.167	0.117	1.420
	17	631-650	70, 69, 52	0.150	0.117	1.278
	18	660-681	18, 7, 17, 51, 50	0.227	0.117	1.937
	19	700-719	41, 10, 4	0.150	0.117	1.278
	20	731-749	16, 49, 68, 3	0.211	0.117	1.794
NIH	1	3 to 12	25, 3	0.200	0.040	5.000
	2	20-43	15, 5, 4, 11, 10	0.208	0.040	5.208
	3*	415-435	18, 13	0.095	0.040	2.381
	4	663-676	1, 6	0.143	0.040	3.571
	5	700-715	30, 7, 3	0.188	0.040	4.688
	6	726-749	27, 9, 26, 24	0.167	0.040	4.167

*Nearby but not overlapping epitopes

Table 43
BIMAS-NIH/Parker algorithm Results for PSA

Rank	Start	Score
1	7	607
2	170	243
3	52	124
4	53	112
5	195	101
6	165	23
7	72	18
8	245	18
9	2	16
10	59	16
11	122	15
12	125	15
13	191	13
14	9	8
15	14	6
16	175	5
17	130	5

Table 44
SYFPEITHI (Rammensee algorithm) Results for PSA

Rank	Start	Score
1	72	26
2	170	22
3	53	22
4	7	22
5	234	21
6	166	21
7	140	21
8	66	21
9	241	20
10	175	20
11	12	20
12	41	19
13	20	19
14	14	19
15	130	18
16	124	18
17	121	18
18	47	18
19	17	18
20	218	17
21	133	17
22	125	17
23	122	17
24	118	17
25	110	17
26	67	17
27	52	17
28	21	17
29	16	17
30	2	17
31	184	16
32	179	16
33	158	16
34	79	16
35	73	16
36	4	16

Table 45

Prediction of clusters for prostate specific antigen (PSA)

Total AAs: 261

Total 9-mers: 253

SYFPEITHI 16: 36 9-mers

NIH 5: 17 9-mers

	Cluster #	AAs	Epitopes (by rank)	Epitopes/AA		
				Cluster	Whole Pr	Ratio
SYFPEITHI	1	2 to 29	30, 36, 4, 11, 14, 29, 19, 13, 28	0.321	0.138	2.330
	2	41-61	12, 18, 27, 3	0.190	0.138	1.381
	3	66-87	8, 26, 1, 35, 34	0.227	0.138	1.648
	4	110-148	25, 24, 17, 23, 16, 22, 15, 21, 7	0.184	0.138	1.332
	5	158-192	33, 6, 2, 10, 32, 31	0.171	0.138	1.243
	6	234-249	5, 9	0.125	0.138	0.906
	7*	118-133	24, 17, 23, 16, 22	0.313	0.138	2.266
	8*	118-138	24, 17, 23, 16, 22, 15	0.286	0.138	2.071
NIH	1	2-22	9, 1, 14, 15	0.190	0.065	2.924
	2	52-67	3, 4, 10	0.188	0.065	2.879
	3	122-138	11, 12, 17	0.176	0.065	2.709
	4	165-183	6, 2, 16	0.158	0.065	2.424
	5	191-203	13, 5	0.154	0.065	2.362
	6**	52-80	3, 4, 10, 7	0.138	0.065	2.118

*These clusters are internal to the less preferred cluster #4.

**Includes a nearby but not overlapping epitope.

Table 46
BIMAS-NIH/Parker algorithm Results for PSCA

Rank	Start	Score
1	43	53
2	5	84
3	7	79
4	109	36
5	105	105
6	108	24
7	14	21
8	20	18
9	115	17
10	42	15
11	36	15
12	99	9
13	58	8

20

Table 47
SYFPEITHI (Rammensee algorithm) Results for PSCA

25

Rank	Start	Score	Rank	Start	Score
1	108	30	17	54	19
2	14	30	18	12	19
3	105	29	19	4	19
4	5	28	20	1	19
5	115	26	21	112	18
6	99	26	22	101	18
7	7	26	23	98	18
8	109	24	24	51	18
9	53	23	25	43	18
10	107	21	26	106	17
11	20	21	27	104	17
12	8	21	28	83	17
13	13	20	29	63	17
14	102	19	30	50	17
15	60	19	31	3	17
16	57	19	32	9	16
			33	92	16

Table 48

Prediction of clusters for prostate stem cell antigen (PSCA)

Total AAs: 123

Total 9-mers: 115

SYFPEITHI 16: 33;

SYFPEITHI 20: 13

NIH 5: 13

	Cluster #	AAs	Epitopes (by rank)	Epitopes/AA		
				Cluster	Whole Pr.	Ratio
SYFPEITHI >16	1	1 to 28	20, 31, 19, 4, 7, 12, 33, 18, 13, 2, 11	0.393	0.268	1.464
	2	43-71	25, 30, 24, 9, 17, 16, 15, 29	0.276	0.268	1.028
	3	92-123	32, 23, 6, 27, 14, 22, 3, 26, 10, 1, 8, 21, 5	0.406	0.268	1.514
SYFPEITHI >20	1	5 to 28	4, 7, 12, 13, 2, 11	0.250	0.106	2.365
	2	99-123	6, 3, 10, 1, 8, 5	0.240	0.106	2.271
NIH	1	5 to 28	2, 3, 7, 8	0.167	0.106	1.577
	2	36-51	11, 10, 1	0.188	0.106	1.774
	3	99-123	12, 5, 6, 4, 9	0.200	0.106	1.892
	4*	105-116	5, 6, 4	0.250	0.106	2.365

*This cluster is internal to the less preferred cluster #3.

In tables 49-60 epitope prediction and cluster analysis data for each algorithm are presented together in a single table.

Table 49

Prediction of clusters for MAGE-1 (NIH algorithm)

Total AAs: 309
Total 9-mers: 301
NIH 5:19 9-mers

Cluster #	AAs	Epitope Rank	Start Position	NIH Score	Cluster	Epitopes/AA Whole Pr.	Ratio
1	18-32	16	18	9	0.133	0.063	2.112
		19	24	7			
2	101-113	14	101	11	0.154	0.063	2.442
		7	105	44			
3	146-159	9	146	32	0.143	0.063	2.263
		3	151	169			
4	169-202	10	169	32	0.176	0.063	2.796
		13	174	16			
		18	181	8			
		17	187	8			
		6	188	74			
		5	194	110			
5	264-277	2	264	190	0.143	0.063	2.263
		12	269	20			
6	278-290	1	278	743	0.154	0.063	2.437
		11	282	28			

Table 50

Prediction of clusters for MAGE-1 (SYFPEITHI algorithm)

Total AAs: 309

Total 9-mers: 301

SYFPEITHI 16: 46 9-mers

Cluster #	Aas	Epitope Rank	Start Position	SYFPEITHI Score	Epitopes/AA		
					Cluster	Whole	Ratio
1	7-49	22	7	19	0.233	0.153	1.522
		9	15	22			
		27	18	18			
		16	20	20			
		28	22	18			
		29	24	18			
		33	31	17			
		30	35	18			
		2	38	26			
		17	41	20			
2	89-132	10	89	22	0.273	0.153	1.783
		18	92	20			
		7	93	23			
		23	96	19			
		43	98	16			
		4	101	25			
		8	105	23			
		34	107	17			
		35	108	17			
		36	113	17			
		37	118	17			
		19	124	20			
3	167-203	44	167	16	0.270	0.153	1.766
		20	169	20			
		12	174	21			
		24	181	19			
		6	187	24			
		31	188	18			
		25	191	19			
		38	192	17			
		1	194	27			
4	230-246	14	230	21	0.118	0.153	0.769
		39	238	17			
5	264-297	15	264	21	0.235	0.153	1.538
		32	269	18			
		40	270	17			
		26	271	19			
		46	275	16			
		3	278	26			
		21	282	20			
		41	289	17			

Table 51

Prediction of clusters for MAGE-2 (NIH algorithm)

Total AAs: 314

Total 9-mers: 308

NIH ≥ 5 : 20 9-mers

Cluster #	AAs	Epitope Rank	Start Position	NIH Score	Cluster	Epitope/AA Whole Pr.	Ratio
1	101-120	18	101	5.373	0.150	0.065	2.310
		16	108	6.756			
		1	112	2800.697			
2	153-167	8	153	31.883	0.200	0.065	3.080
		4	158	168.552			
		7	159	32.138			
3	169-211	14	169	8.535	0.209	0.065	3.223
		19	174	5.346			
		6	176	49.993			
		11	181	15.701			
		15	188	7.536			
		12	195	12.809			
		5	200	88.783			
		10	201	16.725			
4	271-284	3	271	398.324	0.143	0.065	2.200
		9	276	19.658			

Table 52

Prediction of clusters for MAGE-2 (SYFPEITHI algorithm)

Total AAs: 314

Total 9-mers: 308

SYFPEITHI 16: 52 9-mers

Cluster #	AAs	Enitope Rank	Start Position	SYFPEITHI Score	Cluster	Enitopes/AA Whole Pr.	Ratio
1	15-32	13	15	21	0.278	0.169	1.645
		29	18	18			
		43	20	16			
		30	22	18			
		21	24	19			
2	37-56	31	37	18	0.250	0.169	1.481
		16	40	20			
		44	44	16			
		14	45	21			
		22	48	19			
3	96-133	36	96	17	0.211	0.169	1.247
		46	101	16			
		6	108	25			
		47	109	16			
		2	112	27			
		37	120	17			
		38	125	17			
		17	131	20			
4	153-216	12	153	22	0.344	0.169	2.036
		39	158	17			
		7	159	25			
		23	161	19			
		24	162	19			
		48	164	16			
		49	167	16			
		32	170	18			
		50	171	16			
		4	174	26			
		9	176	24			
		51	177	16			
		15	181	21			
		25	188	19			
		18	194	20			
		33	195	18			
		19	198	20			
		3	200	27			
		1	201	28			
		40	202	17			
		10	203	23			
		52	208	16			
5	237-254	26	237	19	0.167	0.169	0.987
		27	245	19			
		34	246	18			
6	271-299	8	271	25	0.241	0.169	1.430
		35	276	18			
		41	277	17			
		11	278	23			
		28	283	19			
		20	285	20			
		42	291	17			

Table 53

Prediction of clusters for MAGE-3 (NIH algorithm)

Total AAs: 314

Total 9-mers: 308

NIH 5: 22 9-mers

Cluster #	AAs	Epitope Rank	Start Position	NIH Score	Cluster	Epitopes/AA Whole Pr.	Ratio
1	101-120	15	101	11.002	0.200	0.071	2.800
		21	105	6.488			
		8	108	49.134			
		2	112	339.313			
2	153-167	18	153	7.776	0.200	0.071	2.800
		6	158	51.77			
		22	159	5.599			
3	174-209	17	174	8.832	0.194	0.071	2.722
		7	176	49.993			
		13	181	15.701			
		19	188	7.536			
		14	195	12.809			
		5	200	88.783			
4	237-251	12	201	16.725	0.200	0.071	2.800
		16	237	10.868			
		4	238	148.896			
5	271-284	20	243	6.88	0.143	0.071	2.000
		1	271	2655.495			
		11	276	19.658			

Table 54

Prediction of clusters for MAGE-3 (SYFPEITHI algorithm)

Total AAs: 314

Total 9-mers: 308

SYFPEITHI 16: 47 9-mers

Cluster #	AAs	Epitope Rank	Start Position	SYFPEITHI Score	Cluster	Epitopes/AA Whole Pr.	Ratio
1	15-32	12	15	21	0.278	0.153	1.820
		26	18	18			
		37	20	16			
		27	22	18			
		18	24	19			
2	38-56	38	38	16	0.263	0.153	1.725
		15	40	20			
		39	44	16			
		13	45	21			
		19	48	19			
3	101-142	28	101	18	0.190	0.153	1.248
		40	105	16			
		1	108	31			
		6	112	25			
		31	120	17			
		32	125	17			
		16	131	20			
		41	134	16			
4	153-216	20	153	19	0.313	0.153	2.048
		29	156	18			
		33	158	17			
		21	159	19			
		34	161	17			
		42	164	16			
		43	167	16			
		10	174	22			
		8	176	23			
		14	181	21			
		22	188	19			
		44	193	16			
		11	194	22			
		23	195	19			
		45	197	16			
		17	198	20			
		3	200	27			
		2	201	28			
		35	202	17			
		46	208	16			
5	220-230	5	220	26	0.182	0.153	1.191
		47	222	16			
6	237-246	7	237	25	0.200	0.153	1.311
		9	238	23			
7	271-293	4	271	27	0.217	0.153	1.425
		30	276	18			
		24	278	19			
		36	283	17			
		25	285	19			

Table 55

Prediction of clusters for PRAME (NIH algorithm)

Total AAs: 509

Total 9-mers: 501

NIH 5: 40 9-mers

Cluster #	AAs	Epitope Rank	Start Position	NIH Score	Cluster	Epitopes/AA Whole Pr.	Ratio
1	33-47	20 17	33 39	18 21	0.133	0.080	1.670
2	71-81	9 32	71 73	50 7	0.2	0.07984	2.505
3	99-108	23 24	100 99	15 13	0.2	0.07984	2.505
4	126-135	38 35	126 127	5 6	0.2	0.07984	2.505
5	224-246	5 8 39	224 230 238	124 63 5	0.130	0.080	1.634
6	290-303	18 14 7	290 292 295	18 23 66	0.214	0.080	2.684
7	305-324	28 30 25 36	305 308 312 316	10 8 13 6	0.200	0.080	2.505
8	394-409	2 12 31	394 397 401	182 42 7	0.188	0.080	2.348
9	422-443	10 3 34 29 4	422 425 431 432 435	49 182 7 9 160	0.227	0.080	2.847
10	459-487	15 11 22 40 37	459 462 466 472 479	21 45 15 5 6	0.172	0.080	2.159

Table 56

Prediction of clusters for PRAME (SYFPEITHI algorithm)

Total AAs: 509

Total 9-mers: 501

SYFPEITHI 17: 80 9-mers

Cluster #	AAs	Epitope Rank	Start Position	SYFPEITHI Score	Cluster	Epitopes/AA Whole Pr.	Ratio
1	18-59	65	18	17	0.238	0.160	1.491
		50	21	18			
		66	26	17			
		35	33	20			
		22	34	22			
		51	37	18			
		5	39	27			
		23	40	22			
		13	44	24			
		46	51	19			
2	78-115	36	78	20	0.263	0.160	1.648
		67	80	17			
		52	84	18			
		24	86	22			
		53	91	18			
		25	93	22			
		9	99	25			
		8	100	26			
		54	103	18			
		55	107	18			
3	191-202	56	191	18	0.167	0.160	1.044
		38	194	20			
4	205-215	26	205	22	0.182	0.160	1.139
		27	207	22			
5	222-238	47	222	19	0.235	0.160	1.474
		14	224	24			
		69	227	17			
		57	230	18			
6	241-273	70	241	17	0.212	0.160	1.328
		15	248	24			
		71	255	17			
		30	258	21			
		39	259	20			
		58	261	18			
7	290-342	40	265	20	0.208	0.160	1.300
		72	290	17			
		48	293	19			
		31	298	21			
		73	301	17			
		18	305	23			
		6	308	27			
		10	312	25			
		19	316	23			
		28	319	22			

Prediction of clusters for PRAME (SYFPEITHI algorithm)

Total AAs: 509

Total 9-mers: 501

SYFPEITHI 17: 80 9-mers

Cluster #	AAs	Epitope Rank	Start Position	SYFPEITHI Score	Cluster	Epitopes/AA Whole Pr.	Ratio
8	343-363	41	326	20	0.238	0.160	1.491
		74	334	17			
		59	343	18			
		60	348	18			
		75	351	17			
		20	353	23			
9	364-447	76	355	17	0.250	0.160	1.566
		49	364	19			
		32	371	21			
		11	372	25			
		61	375	18			
		77	382	17			
		21	390	23			
		78	391	17			
		1	394	30			
		42	397	20			
		62	403	18			
		33	410	21			
		43	418	20			
		34	419	21			
		7	422	27			
		2	425	29			
		79	426	17			
		63	428	18			
		64	431	18			
		12	432	25			
10	455-474	16	435	24	0.200	0.160	1.253
		80	439	17			
		29	455	22			
		17	459	24			
		4	462	28			
		3	466	29			

Table 57
Predication of clusters for CEA (NIH algorithm)

Total AAs:702

Total 9-mers: 694

NIH 5: 30 9-mers

Cluster #	AA	Peptides Rank	Start Position	Score	Cluster	Peptides/AAs Whole Pr.	Ratio
1	17-32	5	17	79.041	0.188	0.043	4.388
		7	18	46.873			
		20	24	12.668			
2	113-129	2	113	167.991	0.118	0.043	2.753
		15	121	21.362			
3	172-187	25	172	9.165	0.125	0.043	2.925
		14	179	27.995			
4	278-291	30	278	5.818	0.143	0.043	3.343
		17	283	19.301			
5	350-365	9	350	43.075	0.125	0.043	2.925
		12	357	27.995			
6	528-543	8	528	43.075	0.125	0.043	2.925
		13	535	27.995			
7	631-645	23	631	9.563	0.200	0.043	4.680
		19	634	13.381			
		24	637	9.245			
8	691-702	1	691	196.407	0.167	0.043	3.900
		27	694	7.769			

Table 58
 Predication of clusters for CEA (SYFPEITHI algorithm)

Total AAs: 702
 Total 9-mers: 694
 SYFPEITHI 16: 81 9-mers

Cluster #	AA	Peptides Rank	Start Position	Score	Cluster	Peptides/AAs Whole Pr.	Ratio
1	5-36	67	5	16	0.250	0.117	2.140
		23	12	19			
		24	16	19			
		9	17	22			
		25	18	19			
		32	19	18			
		68	23	16			
2	37-62	33	28	18	0.269	0.117	2.305
		41	37	17			
		20	44	20			
		26	45	19			
		42	46	17			
		27	50	19			
		43	53	17			
3	99-115	44	54	17	0.235	0.117	2.014
		14	99	21			
		5	100	23			
		45	104	17			
4	116-129	34	107	18	0.143	0.117	1.223
		69	116	16			
		21	121	20			
5	172-187	46	172	17	0.125	0.117	1.070
		70	179	16			
6	192-202	3	192	24	0.182	0.117	1.557
		47	194	17			
7	226-241	48	226	17	0.188	0.117	1.605
		49	229	17			
		15	233	21			
8	307-318	11	307	22	0.250	0.117	2.140
		71	308	16			
		51	310	17			
9	319-349	52	319	17	0.129	0.117	1.105
		53	327	17			
		72	335	16			
		35	341	18			
10	370-388	12	370	22	0.211	0.117	1.802
		54	372	17			
		74	375	16			
		6	380	23			
11	403-419	56	403	17	0.235	0.117	2.014
		57	404	17			
		58	407	17			
		28	411	19			

12	427-442	59	427	17	0.188	0.117	1.605
		75	432	16			
		76	434	16			
13	450-462	77	450	16	0.154	0.117	1.317
		13	454	22			
14	488-505	36	488	18	0.167	0.117	1.427
		18	492	21			
		60	497	17			
15	548-558	4	548	24	0.182	0.117	1.557
		61	550	17			
16	565-577	62	565	17	0.154	0.117	1.317
		19	569	21			
17	579-597	78	579	16	0.143	0.117	1.223
		79	582	16			
		7	589	23			
18	605-618	2	605	25	0.143	0.117	1.223
		38	610	18			
19	631-669	29	631	19	0.154	0.117	1.317
		63	637	17			
		80	644	16			
		64	652	17			
		39	660	18			
		81	661	16			
20	675-702	22	675	20	0.286	0.117	2.446
		30	683	19			
		31	687	19			
		40	688	18			
		65	690	17			
		1	691	31			
		66	692	17			
		8	694	23			

Table 59
Predication of clusters for SCP-1 (NIH algorithm)

Total AAs: 976
 Total 9-mers: 968
 NIH 5: 37 9-mers

Cluster #	AA	Peptides Rank	Start Position	Score	Cluster	Peptides/AAs Whole Pr.	Ratio
1	101-116	15	101	40.589	0.125	0.038	3.270
		13	108	57.255			
2*	281-305	14	281	44.944	0.12	0.038	3.139
		24	288	15.203			
		17	297	32.857			
3	431-447	8	431	80.217	0.073	0.038	1.914
		26	438	11.861			
		4	439	148.896			
4	557-579	11	557	64.335	0.174	0.038	4.550
		19	560	24.937			
		6	564	87.586			
		18	571	32.765			
5	635-650	10	635	69.552	0.125	0.038	3.270
		34	642	6.542			
6	755-767	36	755	5.599	0.154	0.038	4.025
		35	759	5.928			
7	838-854	2	838	284.517	0.118	0.038	3.078
		28	846	11.426			

Table 60
Predication of clusters for SCP-1

Total AAs: 976
 Total 9-mers: 968
 Rammensee 16: 118 9-mers

Cluster #	AA	Peptides Rank	Start Position	Score	Cluster	Peptides/AAs Whole Pr.	Ratio
1	8-28	99	8	16	0.143	0.121	1.182
		77	15	17			
		100	20	16			
2	63-80	78	63	17	0.222	0.121	1.838
		50	66	19			
		102	69	16			
		60	72	18			
3	94-123	79	94	17	0.133	0.121	1.103
		12	101	23			
		17	108	22			
		103	115	16			
4	126-158	35	126	20	0.182	0.121	1.504
		36	133	20			
		51	139	19			
		80	140	17			
		61	143	18			
		37	150	20			
5	161-189	38	161	20	0.207	0.121	1.711
		52	165	19			
		81	171	17			
		82	177	17			
		62	178	18			
		39	181	20			
6	213-230	40	213	20	0.167	0.121	1.379
		13	220	23			
		28	222	21			
7	235-250	63	235	18	0.125	0.121	1.034
		18	242	22			
8	260-296	83	260	17	0.243	0.121	2.012
		105	262	16			
		84	267	17			
		106	269	16			
		41	270	20			
		64	271	18			
		85	274	17			
		19	281	22			
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		30	323	21			
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10	339-355	66	339	18	0.235	0.121	1.946
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 15 361 SMHNALHIYM NGTMSQVQGS ANDPIFLLHH AFVDSIFEQW LRRHRPLQEV
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25

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 35 RKQLVIYEEI
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 15 RPRRTILFAS
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PSMA PROTEIN

30

1: Homo sapiens tyrosinase PubMed, Protein,
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 OCT-2000
 35 DEFINITION Homo sapiens tyrosinase (oculocutaneous albinism IA)
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 40 ORGANISM Homo sapiens
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 Euteleostomi;
 Mammalia; Eutheria; Primates; Catarrhini; Hominidae;
 Homo.
 45 REFERENCE 1 (bases 1 to 1964)
 AUTHORS Kwon BS, Haq AK, Pomerantz SH and Halaban R.
 TITLE Isolation and sequence of a cDNA clone for human
 tyrosinase that
 maps at the mouse c-albino locus
 50 JOURNAL Proc. Natl. Acad. Sci. U.S.A. 84 (21), 7473-7477
 (1987)
 MEDLINE 88041128
 PUBMED 2823263
 REMARK Erratum: [[published erratum appears in Proc Natl Acad
 55 Sci U S A

- 1988 Sep;85(17):6352]]
 2 (bases 1 to 1964)
 REFERENCE
 AUTHORS Barton DE, Kwon BS and Francke U.
 TITLE Human tyrosinase gene, mapped to chromosome 11 (q14---
 5 -q21),
 defines second region of homology with mouse
 chromosome 7
 JOURNAL Genomics 3 (1), 17-24 (1988)
 MEDLINE 89122007
 10 PUBMED 3146546
 REFERENCE 3 (bases 181 to 1964)
 AUTHORS Shibahara,S., Tomita,Y., Tagami,H., Muller,R.M. and
 Cohen,T.
 15 TITLE Molecular basis for the heterogeneity of human
 tyrosinase
 JOURNAL Tohoku J. Exp. Med. 156 (4), 403-414 (1988)
 MEDLINE 89222868
 REFERENCE 4 (bases 1 to 1964)
 20 AUTHORS Bouchard B, Fuller BB, Vijayasaradhi S and Houghton
 AN.
 TITLE Induction of pigmentation in mouse fibroblasts by
 expression of
 human tyrosinase cDNA
 JOURNAL J. Exp. Med. 169 (6), 2029-2042 (1989)
 25 MEDLINE 89279151
 PUBMED 2499655
 REFERENCE 5 (bases 1 to 1964)
 AUTHORS Takeda,A., Tomita,Y., Okinaga,S., Tagami,H. and
 Shibahara,S.
 30 TITLE Functional analysis of the cDNA encoding human
 tyrosinase precursor
 JOURNAL Biochem. Biophys. Res. Commun. 162 (3), 984-990 (1989)
 MEDLINE 89351001
 REFERENCE 6 (bases 1 to 1964)
 35 AUTHORS Kikuchi H, Miura H, Yamamoto H, Takeuchi T, Dei T and
 Watanabe M.
 TITLE Characteristic sequences in the upstream region of the
 human
 tyrosinase gene
 40 JOURNAL Biochim. Biophys. Acta 1009 (3), 283-286 (1989)
 MEDLINE 90089403
 PUBMED 2480811
 REFERENCE 7 (bases 1 to 1964)
 45 AUTHORS Giebel LB, Strunk KM and Spritz RA.
 TITLE Organization and nucleotide sequences of the human
 tyrosinase gene
 and a truncated tyrosinase-related segment
 JOURNAL Genomics 9 (3), 435-445 (1991)
 50 MEDLINE 91236163
 PUBMED 1903356
 REFERENCE 8 (bases 1 to 1964)
 55 AUTHORS Brichard V, Van Pel A, Wolfel T, Wolfel C, De Plaen E,
 Lethe B,
 Coulie P and Boon T.
 TITLE The tyrosinase gene codes for an antigen recognized by
 autologous
 cytolytic T lymphocytes on HLA-A2 melanomas
 JOURNAL J. Exp. Med. 178 (2), 489-495 (1993)

MEDLINE 93340625
 PUBMED 8340755
 COMMENT PROVISIONAL REFSEQ: This record has not yet been
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FEATURES

source Location/Qualifiers
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 10 ctatttggtta
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	1: Homo sapiens synovial sarcoma, NM_003147 X breakpoint 2 (SSX2), mRNA	PubMed, Protein, Related Sequences, Taxonomy, OMIM, LinkOut
15	LOCUS NM_003147 766 bp mRNA MAR-2001 DEFINITION Homo sapiens synovial sarcoma, X breakpoint 2 (SSX2), mRNA. ACCESSION NM_003147 20 VERSION NM_003147.1 GI:10337582 KEYWORDS . SOURCE human. ORGANISM Homo sapiens Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; 25 Euteleostomi; Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo. REFERENCE 1 (bases 1 to 766) AUTHORS Shipley JM, Clark J, Crew AJ, Birdsall S, Rocques PJ, 30 Gill S, Chelly J, Monaco AP, Abe S, Gusterson BA and et al. TITLE The t(X;18)(p11.2;q11.2) translocation found in human synovial sarcomas involves two distinct loci on the X 35 chromosome JOURNAL Oncogene 9 (5), 1447-1453 (1994) MEDLINE 94203675 PUBMED 8152806 REFERENCE 2 (bases 1 to 766) 40 AUTHORS Crew,A.J., Clark,J., Fisher,C., Gill,S., Grimer,R., Chand,A., Shipley,J., Gusterson,B.A. and Cooper,C.S. TITLE Fusion of SYT to two genes, SSX1 and SSX2, encoding 45 proteins with homology to the Kruppel-associated box in human synovial sarcoma JOURNAL EMBO J. 14 (10), 2333-2340 (1995) MEDLINE 95292974 REFERENCE 3 (bases 1 to 766) 50 AUTHORS Tureci O, Sahin U, Schobert I, Koslowski M, Scmitt H, Schild HJ, Stenner F, Seitz G, Rammensee HG and Pfreundschuh M. TITLE The SSX-2 gene, which is involved in the t(X;18) translocation of	

synovial sarcomas, codes for the human tumor antigen

HOM-MEL-40
 JOURNAL Cancer Res. 56 (20), 4766-4772 (1996)
 MEDLINE 96438636
 PUBMED 8840996

5 COMMENT PROVISIONAL REFSEQ: This record has not yet been
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X86175.1.

10 FEATURES Location/Qualifiers
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1: **Homo sapiens folate hydrolase** PubMed, Protein, Related
NM_004 (prostate-specific membrane antigen) Sequences, Taxonomy, OMIM,
476 1 (FOLH1), mRNA LinkOut
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 NOV-2000
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 25 Mammalia; Eutheria; Primates; Catarrhini; Hominidae;
 Homo.
 REFERENCE 1 (bases 1 to 2653)
 AUTHORS Israeli,R.S., Powell,C.T., Fair,W.R. and Heston,W.D.
 30 TITLE Molecular cloning of a complementary DNA encoding a
 prostate-specific membrane antigen
 JOURNAL Cancer Res. 53 (2), 227-230 (1993)
 MEDLINE 93113576
 REFERENCE 2 (bases 1 to 2653)
 35 AUTHORS Rinker-Schaeffer CW, Hawkins AL, Su SL, Israeli RS,
 Griffin CA,
 Isaacs JT and Heston WD.
 TITLE Localization and physical mapping of the prostate-
 specific membrane
 40 antigen (PSM) gene to human chromosome 11
 JOURNAL Genomics 30 (1), 105-108 (1995)
 MEDLINE 96129312
 PUBMED 8595888
 REFERENCE 3 (bases 1 to 2653)
 45 AUTHORS O'Keefe DS, Su SL, Bacich DJ, Horiguchi Y, Luo Y,
 Powell CT,
 Zandvliet D, Russell PJ, Molloy PL, Nowak NJ, Shows
 TB, Mullins C,
 Vonder Haar RA, Fair WR and Heston WD.
 50 TITLE Mapping, genomic organization and promoter analysis of
 the human
 prostate-specific membrane antigen gene
 JOURNAL Biochim. Biophys. Acta 1443 (1-2), 113-127 (1998)
 MEDLINE 99057588
 PUBMED 9838072
 55 REFERENCE 4 (bases 1 to 2653)

AUTHORS Maraj BH, Leek JP, Karayi M, Ali M, Lench NJ and
 Markham AF.
 TITLE Detailed genetic mapping around a putative prostate-
 specific
 5 membrane antigen locus on human chromosome 11p11.2
 JOURNAL Cytogenet. Cell Genet. 81 (1), 3-9 (1998)
 MEDLINE 98358137
 PUBMED 9691167
 COMMENT PROVISIONAL REFSEQ: This record has not yet been
 10 subject to final
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Homo.
REFERENCE 1 (bases 1 to 2817)
AUTHORS Kim,K.K., Youn,B.S., Heng,H.H., Shi,X.M., Tsui,L.C.,
Lee,Z.H.,
20 Pickard,R.T. and Kwon,B.S.
TITLE Genomic organization and FISH mapping of human Pmel
17, the
putative silver locus
JOURNAL Pigment Cell Res. 9 (1), 42-48 (1996)
25 MEDLINE 96314705
REFERENCE 2 (bases 1 to 2817)
AUTHORS Kwon,B.S.
TITLE Direct Submission
JOURNAL Submitted (05-JAN-1995) Indiana University School of
30 Medicine,
Microbiology and Immunology, 635 Barnhill Drive,
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 15 REFERENCE 1 (bases 1 to 1466)
 AUTHORS Lundwall,A. and Lilja,H.
 TITLE Molecular cloning of human prostate specific antigen
 cDNA
 JOURNAL FEBS Lett. 214 (2), 317-322 (1987)
 20 MEDLINE 87190978
 REFERENCE 2 (bases 1 to 1466)
 AUTHORS Sutherland GR, Baker E, Hyland VJ, Callen DF, Close JA,
 Tregear GW,
 Evans BA and Richards RI.
 25 TITLE Human prostate-specific antigen (APS) is a member of
 the glandular
 kallikrein gene family at 19q13
 JOURNAL Cytogenet. Cell Genet. 48 (4), 205-207 (1988)
 30 MEDLINE 89250658
 PUBMED 2470553
 REFERENCE 3 (bases 1 to 1466)
 AUTHORS Riegman PH, Klaassen P, van der Korput JA, Romijn JC
 and Trapman J.
 35 TITLE Molecular cloning and characterization of novel
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 JOURNAL Biochem. Biophys. Res. Commun. 155 (1), 181-188 (1988)
 MEDLINE 88326297
 40 PUBMED 2458104
 REFERENCE 4 (bases 1 to 1466)
 AUTHORS Schulz P, Stucka R, Feldmann H, Combriato G, Klobeck HG
 and Fittler F.
 TITLE Sequence of a cDNA clone encompassing the complete
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 45 prostate specific antigen (PSA) and an unspliced
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 JOURNAL Nucleic Acids Res. 16 (13), 6226 (1988)
 MEDLINE 88289366
 50 PUBMED 2456523
 REFERENCE 5 (bases 1 to 1466)
 AUTHORS Riegman PH, Vlietstra RJ, van der Korput JA, Romijn JC
 and Trapman J.
 TITLE Characterization of the prostate-specific antigen gene:
 a novel
 55 human kallikrein-like gene
 JOURNAL Biochem. Biophys. Res. Commun. 159 (1), 95-102 (1989)
 MEDLINE 89165891
 PUBMED 2466464

REFERENCE 6 (bases 1 to 1466)
 AUTHORS Henttu P and Vihko P.
 TITLE cDNA coding for the entire human prostate specific
 antigen shows high homologies to the human tissue kallikrein
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 JOURNAL Biochem. Biophys. Res. Commun. 160 (2), 903-910 (1989)
 MEDLINE 89246551
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 REFERENCE 7 (bases 1 to 1466)
 AUTHORS Klobeck HG, Combriato G, Schulz P, Arbusow V and
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 TITLE Genomic sequence of human prostate specific antigen
 (PSA)
 JOURNAL Nucleic Acids Res. 17 (10), 3981 (1989)
 MEDLINE 89282407
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 REFERENCE 8 (bases 1 to 1466)
 AUTHORS Lundwall A.
 TITLE Characterization of the gene for prostate-specific
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 JOURNAL Biochem. Biophys. Res. Commun. 161 (3), 1151-1159
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	241	catcaggaac	aaaagcgtga	tcttgctggg	tcggcacagc	ctgtttcatc
	301	aggccaggta	tttcagggtca	gccacagctt	cccacaccog	ctctacgata
15	361	gaagaatcga	ttcctcaggc	caggtgatga	ctccagccac	gacctcatgc
	421	gtcagagcct	gccgagctca	cggatgctgt	gaaggtcatg	gacctgcca
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	541	gaccccaaag	aaacttcagt	gtgtggacct	ccatgttatt	tccaatgacg
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25	661	aagcacctgc	tcgggtgatt	ctgggggccc	acttgtctgt	aatggtgtgc
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30	781	ggtgcattac	cggaagtgga	tcaaggacac	catcgtggcc	aaccctgag
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	1021	ctggggaata	ctggccatgc	ctggagacat	atcactcaat	ttctctgagg
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	1141	agagagtgga	gagtgacatg	tgctggacac	tgtccatgaa	gactgagca
	1201	gcacaacgca	ccagacactc	acagcaagga	tggagctgaa	aacataaccc
45	1261	ggaggcactg	ggaagcctag	agaaggctgt	gagccaagga	gggagggtct
	1321	tgggatgggg	atgaagtaag	gagagggact	ggacccctg	gaagctgatt
50	1381	ggaggtgtat	tgaagtcctc	cagacaaccc	tcagatttga	tgatttccta
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55	U87459. Human autoimmunog... [gi:1890098]					
	LOCUS	HSU87459	752 bp	mRNA		PRI
	22-DEC-1999					

DEFINITION Human autoimmunogenic cancer/testis antigen NY-ESO-1 mRNA, complete cds.
 ACCESSION U87459
 VERSION U87459.1 GI:1890098
 5 KEYWORDS .
 SOURCE human.
 ORGANISM Homo sapiens
 Eukaryota; Metazoa; Chordata; Craniata;
 Vertebrata; Euteleostomi;
 10 Mammalia; Eutheria; Primates; Catarrhini;
 Hominidae; Homo.
 REFERENCE 1 (bases 1 to 752)
 AUTHORS Chen,Y.T., Scanlan,M.J., Sahin,U., Tureci,O.,
 Gure,A.O., Tsang,S.,
 15 Williamson,B., Stockert,E., Pfreundschuh,M. and
 Old,L.J.
 TITLE A testicular antigen aberrantly expressed in human
 cancers detected
 by autologous antibody screening
 20 JOURNAL Proc. Natl. Acad. Sci. U.S.A. 94 (5), 1914-1918 (1997)
 MEDLINE 97203161
 PUBMED 9050879
 REFERENCE 2 (bases 1 to 752)
 AUTHORS Chen,Y.-T.
 25 TITLE Direct Submission
 JOURNAL Submitted (28-JAN-1997) Ludwig Institute for Cancer
 Research, New
 York Branch, 1275 York Avenue, New York, NY 10021,
 USA
 30 FEATURES Location/Qualifiers
 source 1..752
 /organism="Homo sapiens"
 /db_xref="taxon:9606"
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 35 carcinoma expression
 cdna library"
 CDS 54..596
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 40 antigen NY-ESO-1"
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 BASE COUNT 126 a 230 c 256 g 140 t
 ORIGIN
 50 1 atcctcgtgg gccctgacct tctctctgag agccgggcag aggcctccgga
 gccatgcagg
 61 ccgaaggccg gggcacaggg ggttcgacgg gcgatgctga tggcccagga
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 gccccgcggg

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      241 gtccgcatgg cggcgcggtc tcagggctga atggatgctg cagatgcggg
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atggaagcag
5      361 agctggcccg caggagcctg gcccaggatg cccacccgct tcccgtgcca
ggggtgcttc
      421 tgaaggagtt cactgtgtcc ggcaacatac tgactatccg actgactgct
gcagaccacc
      481 gccaaactgca gctctccatc agctcctgtc tccagcagct ttccctgttg
10 atgtgggatca
      541 cgcagtgcctt tctgcccgtg tttttggctc agcctccctc agggcagagg
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15      661 gccagttcat tgtggggggc tgattgtttg tcgctggagg aggacggctt
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20 CAA11116. LAGE-1a protein [...[gi:3255959]
LOCUS CAA11116 180 aa PRI
23-JUN-1998
DEFINITION LAGE-1a protein [Homo sapiens].
ACCESSION CAA11116
25 PID g3255959
VERSION CAA11116.1 GI:3255959
DBSOURCE embl locus HOS223093, accession AJ223093.1
KEYWORDS .
SOURCE human.
30 ORGANISM Homo sapiens
Eukaryota; Metazoa; Chordata; Craniata;
Vertebrata; Euteleostomi;
Mammalia; Eutheria; Primates; Catarrhini;
Hominidae; Homo.
35 REFERENCE 1 (residues 1 to 180)
AUTHORS Lethe,B., Lucas,S., Michaux,L., De Smet,C.,
Godelaine,D.,
Serrano,A., De Plaen,E. and Boon,T.
40 TITLE LAGE-1, a new gene with tumor specificity
JOURNAL Int. J. Cancer 76 (6), 903-908 (1998)
MEDLINE 98289662
REFERENCE 2 (residues 1 to 180)
AUTHORS Lethe,B.G.
45 TITLE Direct Submission
JOURNAL Submitted (08-JAN-1998) Lethe B.G., Brussels Branch,
Ludwig
Institute for Cancer Research, 74 Avenue
Hippocrate, B - 1200 -
Bruxelles, BELGIUM
50 COMMENT Related sequences: AJ223040, AJ223041 and AJ003149.
FEATURES Location/Qualifiers
source 1..180
/organism="Homo sapiens"
55 /isolate="individual LB33"
/db_xref="taxon:9606"
/chromosome="X"
/map="q28"
/clone="c2RB"

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                                /sex="Female"
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5      Protein      1..180
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                                /gene="LAGE-1"
                                /db_xref="SPTREMBL:O75637"
10     /coded_by="join(AJ223093.1:1231..1499,
AJ223093.1:2115..2249,AJ223093.1:2479..2617)"
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15     1  mqaegrgtgg stgdadgpgg pgipdgpggn aggpgeagat ggrgprgaga
arasgprgga
      61  prgphggaas aqdgrcpcga rrpdsrllel hitmpfsspm eaelvrrils
rdaaplprpg
      121 avlkdfvtsg nllfirltaa dhrqlqlsis sclqqlslm witqcflpvf
laqapsqgrr
20     181
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CAA11117. LAGE-1b protein [...[gi:3255960]
LOCUS      CAA11117      210 aa      PRI
25  23-JUN-1998
DEFINITION LAGE-1b protein [Homo sapiens].
ACCESSION  CAA11117
PID        g3255960
VERSION    CAA11117.1  GI:3255960
30  DBSOURCE  embl locus HOS223093, accession AJ223093.1
KEYWORDS
SOURCE     human.
ORGANISM   Homo sapiens
          Eukaryota; Metazoa; Chordata; Craniata;
35  Vertebrata; Euteleostomi;
          Mammalia; Eutheria; Primates; Catarrhini;
Hominidae; Homo.
REFERENCE  1 (residues 1 to 210)
AUTHORS   Lethe,B., Lucas,S., Michaux,L., De Smet,C.,
40  Godelaine,D.,
          Serrano,A., De Plaen,E. and Boon,T.
TITLE     LAGE-1, a new gene with tumor specificity
JOURNAL   Int. J. Cancer 76 (6), 903-908 (1998)
MEDLINE   98289662
45  REFERENCE 2 (residues 1 to 210)
AUTHORS   Lethe,B.G.
TITLE     Direct Submission
JOURNAL   Submitted (08-JAN-1998) Lethe B.G., Brussels Branch,
50  Ludwig
          Institute for Cancer Research, 74 Avenue
Hippocrate, B - 1200 -
          Bruxelles, BELGIUM
COMMENT   Related sequences: AJ223040, AJ223041 and AJ003149.
FEATURES   Location/Qualifiers
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              /isolate="individual LB33"
              /db_xref="taxon:9606"

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                    /clone="c2RB"
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                    /cell_type="melanoma"
                    /clone_lib="B6"
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10                CDS 1..210
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                arasgprgga
18                61 prgphggaas aqdgrcpcga rrpdsrllel hitmpfsspm eaelvrrils
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25                //

30                M77481. Human antigen (MA...[gi:416114]
                LOCUS HUMMAG1A 2420 bp DNA PRI
                15-NOV-1993
                DEFINITION Human antigen (MAGE-1) gene, complete cds.
                ACCESSION M77481
35                VERSION M77481.1 GI:416114
                KEYWORDS antigen.
                SOURCE Homo sapiens (individual_isolate patient MZ2)
                melanoma abdominal
                    metastasis of melanoma DNA.
40                ORGANISM Homo sapiens
                    Eukaryota; Metazoa; Chordata; Craniata;
                Vertebrata; Euteleostomi;
                    Mammalia; Eutheria; Primates; Catarrhini;
                Hominidae; Homo.
45                REFERENCE 1 (bases 785 to 1286)
                AUTHORS van der Bruggen,P., Traversari,C., Chomez,P.,
                Lurquin,C., De
                    Plaen,E., Van den Eynde,B., Knuth,A. and Boon,T.
50                TITLE A gene encoding an antigen recognized by cytolytic T
                lymphocytes on
                    a human melanoma
                JOURNAL Science 254, 1643-1647 (1991)
                MEDLINE 92086861
                REFERENCE 2 (bases 1 to 2420)
55                AUTHORS van der Bruggen P.
                TITLE Direct Submission
                JOURNAL Submitted (05-FEB-1992) Pierre van der Bruggen, Ludwig
                Institute

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for Cancer Research, Brussels Branch, Avenue
Hippocrate, 74, UCL
7459, Brussels, B-1200, Belgium

COMMENT On Nov 15, 1993 this sequence version replaced
gi:187294.

5 FEATURES Location/Qualifiers

source 1..2420
/organism="Homo sapiens"
/db_xref="taxon:9606"

10 /map="X"
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15 /gene="MAGE-1"
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exon 413..485
/gene="MAGE-1"
/number=2

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intron 486..560
/gene="MAGE-1"

25 /number=2

exon 561..2111
gene="MAGE-1"
/number=3

30 gene 626..1555
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CDS 626..1555
/gene="MAGE-1"
/codon_start=1
/product="MAGE-1"

35 /protein_id="AAA03229.1"
/db_xref="GI:416115"

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40 SCILESLEFRAVITKKVADLVGFLLKLRAREPVTKAEMLESVIKNYKHCFPE
IFGKASESLQLVFGIDVKEADPTGHSYVLVTCLGLSYDGLLDGNQIMPKTGF
LIIVLVMIAMEGGHAPEEIIWEELSVMEVYDGREHSAYGEPRKLLTQDLVQE
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45 polyA_signal 2095..2100
/gene="MAGE-1"

intron 2112..2420
/partial
gene="MAGE-1"

50 /number=3

BASE COUNT 562 a 582 c 677 g 599 t
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gggggtcatcc

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5	301	caggacacat	aggactccac	agagtctggc	ctcacctccc	tactgtcagt
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		gggcctctgc				
	1441	tgaaaccagc	tatgtgaaag	tccttgagta	tgtgatcaag	gtcagtgcaa
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    aaagatgtag
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    gagctgattg
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20    L18920. Human MAGE-2 gene...[gi:436180]
    LOCUS          HUMMAGE2X      4559 bp      DNA          PRI
    20-APR-1994
    DEFINITION     Human MAGE-2 gene exons 1-4, complete cds.
    ACCESSION      L18920
25    VERSION       L18920.1  GI:436180
    KEYWORDS       .
    SOURCE          Homo sapiens (human).
    ORGANISM       Homo sapiens
                                Eukaryota;      Metazoa;      Chordata;      Craniata;
30    Vertebrata; Euteleostomi;
                                Mammalia;      Eutheria;      Primates;      Catarrhini;
                                Hominidae; Homo.
    REFERENCE      1 (bases 1 to 4559)
    AUTHORS        De Smet,C., Lurquin,C., van der Bruggen,P., De
35    Plaen,E.,
                                Brasseur,F. and Boon,T.
    TITLE          Sequence and expression pattern of the human MAGE2 gene
    JOURNAL         Immunogenetics 39 (2), 121-129 (1994)
    MEDLINE        94102805
40    FEATURES      Location/Qualifiers
        source      1..4559
                                /organism="Homo sapiens"
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                                /db_xref="taxon:9606"
45                                /map="X"
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                                /sex="female"
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                                /cell_type="melanoma cell line"
50        gene      561..4496
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        exon      1851..1969

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intron            1970..2788
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intron            2855..2934
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exon              2935..4496
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LGEVPAADSPSPPHSPQGASSFSTTINYTLWRQSDGSSNQEEGPRMFPDLE
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25 EVFEGREDSVFAHPRKLIMQDLVQENYLEYRQVPGSDPACYEFLWGPRLIETSYVKVLHHTL
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gactgaggat
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35    121 acacactggc aatgatctca ccccgagcac acccctcccc ccaatgccac
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45    421 atccggttcc acccttgccg tgaaccagg gaagtcacgg gcccgatgt
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55    721 accccccgcc gctttaaccg cagggaactc tggcgtaaga gctttgtgtg
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5	961	ccaccatcct	ggcagaatcc	ggctttgccc	ctgcaatcaa	cccacggaag
		ctccgggaat				
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      REFERENCE 1 (bases 1 to 4204)
25      AUTHORS Gaugler,B., Van den Eynde,B., van der Bruggen,P.,
      Romero,P.,
      Gaforio,J.J., De Plaen,E., Lethe,B., Brasseur,F.
      and Boon,T.
      TITLE      Human gene MAGE-3 codes for an antigen recognized on
30      a melanoma by
      autologous cytolytic T lymphocytes
      JOURNAL J. Exp. Med. 179, 921-930 (1994)
      MEDLINE 94157413
      REFERENCE 2 (bases 1 to 4204)
35      AUTHORS Gaugler,B.
      TITLE      Direct Submission
      JOURNAL Submitted (25-NOV-1993) Beatrice Gaugler, Ludwig
      Institute for
      Cancer Research, 74 Avenue Hippocrate, Brussels
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      REFERENCE  1 (bases 1 to 990)

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AUTHORS Reiter,R.E., Gu,Z., Watabe,T., Thomas,G., Kinga,S.,
 Davis,E., Wahl,M., Nisitani,S., Yamashiro,J., Le Beau,M.M.,
 5 Losa,M. and Witte,O.N.
 TITLE Prostate stem cell antigen: a cell surface marker
 overexpressed in prostate cancer
 JOURNAL Proc. Natl. Acad. Sci. U.S.A. 95 (4), 1735-1740 (1998)
 10 MEDLINE 98132661
 REFERENCE 2 (bases 1 to 990)
 AUTHORS Reiter,R.E.
 TITLE Direct Submission
 JOURNAL Submitted (19-JAN-1998) Urology, UCLA, 66-134 CHS
 15 10833 Le Conte
 Ave., Los Angeles, CA 90095, USA
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      extra accessions:Q9UMJ1,created: Jan 1, 1988.
      sequence updated: Jan 1, 1988.
      annotation updated: Aug 20, 2001.
30      xrefs: gi: gi: 186652, gi: gi: 186653, gi: gi: 186649,
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      186651, gi: gi: 186645, gi: gi: 186646, gi: gi:
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35      gi: gi:
      386843, gi: gi: 67558
      xrefs (non-sequence databases): HSSP P00757, MEROPS
S01.160,
      GlycoSuiteDB P06870, MIM 147910, InterPro IPR001314,
40      InterPro
      IPR001254, Pfam PF00089, PRINTS PR00722, PROSITE
PS50240, PROSITE
      PS00134, PROSITE PS00135
KEYWORDS      Hydrolase; Serine protease; Glycoprotein; Multigene
45      family;
      Zymogen; Signal.
SOURCE      human.
ORGANISM      Homo sapiens
      Eukaryota; Metazoa; Chordata; Craniata; Vertebrata;
50      Euteleostomi;
      Mammalia; Eutheria; Primates; Catarrhini; Hominidae;
      Homo.
REFERENCE      1 (residues 1 to 262)
AUTHORS      Fukushima,D., Kitamura,N. and Nakanishi,S.
55      TITLE      Nucleotide sequence of cloned cDNA for human
      pancreatic kallikrein
JOURNAL      Biochemistry 24, 8037-8043 (1985)
REMARK      SEQUENCE FROM N.A.

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TISSUE=Pancreas
 REFERENCE 2 (residues 1 to 262)
 AUTHORS Evans,B.A., Yun,Z.X., Close,J.A., Tregear,G.W.,
 Kitamura,N.,
 5 Nakanishi,S., Callen,D.F., Baker,E., Hyland,V.J.,
 Sutherland,G.R.
 and Richards,R.I.
 TITLE Structure and chromosomal localization of the human
 renal
 10 kallikrein gene
 JOURNAL Biochemistry. 27 (9), 3124-3129 (1988)
 MEDLINE 88269498
 PUBMED 2898948
 REMARK SEQUENCE FROM N.A.
 15 TISSUE=Kidney
 REFERENCE 3 (residues 1 to 262)
 AUTHORS Angermann,A., Bergmann,C. and Appelhans,H.
 TITLE Cloning and expression of human salivary-gland
 kallikrein in
 20 Escherichia coli
 JOURNAL The Biochemical journal. 262 (3), 787-793 (1989)
 MEDLINE 90073574
 PUBMED 2686621
 REMARK SEQUENCE FROM N.A.
 25 TISSUE=Salivary gland
 REFERENCE 4 (residues 1 to 262)
 AUTHORS Baker,A.R. and Shine,J.
 TITLE Human kidney kallikrein: cDNA cloning and sequence
 analysis
 30 JOURNAL DNA (Mary Ann Liebert, Inc.) 4 (6), 445-450 (1985)
 MEDLINE 86135264
 PUBMED 3853975
 REMARK SEQUENCE OF 17-262 FROM N.A.
 TISSUE=Kidney
 35 REFERENCE 5 (residues 1 to 262)
 AUTHORS Lu,H.S., Lin,F.K., Chao,L. and Chao,J.
 TITLE Human urinary kallikrein. Complete amino acid sequence
 and sites of
 glycosylation
 40 JOURNAL International journal of peptide and protein research.
 33 (4),
 237-249 (1989)
 MEDLINE 89326688
 PUBMED 2666327
 45 REMARK SEQUENCE OF 25-262.
 TISSUE=Urine
 REFERENCE 6 (residues 1 to 262)
 AUTHORS Kellermann,J., Lottspeich,F., Geiger,R. and
 Deutzmann,R.
 50 TITLE Human urinary kallikrein--amino acid sequence and
 carbohydrate
 attachment sites
 JOURNAL Protein sequences & data analysis. 1 (3), 177-182
 (1988)
 55 MEDLINE 88203586
 PUBMED 3163150
 REMARK SEQUENCE OF 25-262, AND CARBOHYDRATE-LINKAGE SITES.
 TISSUE=Urine

REFERENCE 7 (residues 1 to 262)
 AUTHORS Lottspeich,F., Geiger,R., Henschen,A. and Kutzbach,C.
 TITLE N-Terminal amino acid sequence of human urinary
 kallikrein homology
 5 with other serine proteases
 JOURNAL Hoppe-Seyler's Zeitschrift fur physiologische Chemie.
 360 (12), 1947-1950 (1979)
 MEDLINE 80114126
 10 PUBMED 393608
 REMARK SEQUENCE OF 25-55.
 TISSUE=Urine
 REFERENCE 8 (residues 1 to 262)
 AUTHORS Takahashi,S., Irie,A., Katayama,Y., Ito,K. and
 15 Miyake,Y.
 TITLE N-terminal amino acid sequence of human urinary
 prokallikrein
 JOURNAL Journal of biochemistry. 99 (3), 989-992 (1986)
 MEDLINE 86223893
 20 PUBMED 3635530
 REMARK SEQUENCE OF 28-47.
 TISSUE=Urine
 [FUNCTION] GLANDULAR KALLIKREINS CLEAVE MET-LYS AND
 ARG-SER BONDS
 25 IN KININOGEN TO RELEASE LYS-BRADYKININ.
 [CATALYTIC ACTIVITY] PREFERENTIAL CLEAVAGE OF ARG-|-
 XAA BONDS IN
 SMALL MOLECULE SUBSTRATES. HIGHLY SELECTIVE ACTION TO
 RELEASE
 30 KALLIDIN (LYSYL-BRADYKININ) FROM KININOGEN INVOLVES
 HYDROLYSIS OF
 MET-|-XAA OR LEU-|-XAA.
 [SIMILARITY] BELONGS TO PEPTIDASE FAMILY S1; ALSO
 KNOWN AS THE
 35 TRYPSIN FAMILY. KALLIKREIN SUBFAMILY.
 FEATURES Location/Qualifiers
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 /organism="Homo sapiens"
 /db_xref="taxon:9606"
 40 1..262
 Protein 1..262
 /product="GLANDULAR KALLIKREIN 1 PRECURSOR"
 /EC_number="3.4.21.35"
 Region 1..18
 45 /region_name="Signal"
 /note="PROBABLE."
 Region 19..24
 /region_name="Propeptide"
 /note="ACTIVATION PEPTIDE (PROBABLE)."
 50 Region 25..262
 /region_name="Mature chain"
 /note="GLANDULAR KALLIKREIN 1."
 Bond bond(31,174)
 /bond_type="disulfide"
 55 Bond bond(50,66)
 /bond_type="disulfide"
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Site      93
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           /note="O-LINKED."
Site      102
           /site_type="glycosylation"
           /note="N-LINKED (GLCNAC...)."
10         Site      104
           /site_type="glycosylation"
           /note="O-LINKED."
Site      108
           /site_type="glycosylation"
           /note="N-LINKED (GLCNAC...)."
15         Site      120
           /site_type="active"
           /note="CHARGE RELAY SYSTEM."
Region    145
20         /region_name="Variant"
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Bond      bond(153,220)
           /bond_type="disulfide"
           /note="BY SIMILARITY."
25         Site      165
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           /note="N-LINKED (GLCNAC...) (PARTIAL)."
Site      167
           /site_type="glycosylation"
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Region    186
35         /region_name="Variant"
           /note="E -> K. /FTId=VAR_006626."
Bond      bond(210,235)
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           /note="BY SIMILARITY."
40         Site      214
           /site_type="active"
           /note="CHARGE RELAY SYSTEM."
ORIGIN
45         1 mwflvlclal slggtgaapp iqsrivggwe ceqhsqpwqa alyhfstfqc
           ggilvhrqvw
           61 ltaahcisdn yqlwlgrhnl fddentaqfv hvsesfphpg fnmsllenht
           rqadedyshd
           121 lmlrlrtepa dtitdavkvv elptqepevg stclasgwgs iepenfspdp
           dlqcvdlkil
50         181 pndecekahv qkvtdfmlcv ghleggkdtc vgdsggplmc dgvlqgvtsw
           gyvpcgtpnk
           241 psvavrvlsy vkwiedtia ns
           //
55
P08217. ELASTASE 2A PRECU...[gi:119255]
LOCUS      EL2A_HUMAN      269 aa      PRI      20-
AUG-2001

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DEFINITION ELASTASE 2A PRECURSOR.
 ACCESSION P08217
 PID g119255
 VERSION P08217 GI:119255
 5 DBSOURCE swissprot: locus EL2A_HUMAN, accession P08217;
 class: standard.
 created: Aug 1, 1988.
 sequence updated: Aug 1, 1988.
 annotation updated: Aug 20, 2001.
 10 xrefs: gi: gi: 182022, gi: gi: 182023, gi: gi: 182057,
 gi: gi:
 182058, gi: gi: 88298, gi: gi: 88299
 xrefs (non-sequence databases): MEROPS S01.155,
 InterPro IPR001314,
 15 InterPro IPR001254, Pfam PF00089, PRINTS PR00722,
 PROSITE PS50240,
 PROSITE PS00134, PROSITE PS00135
 KEYWORDS Hydrolase; Serine protease; Pancreas; Zymogen; Signal.
 SOURCE human.
 20 ORGANISM Homo sapiens
 Euteleostomi;
 Mammalia; Eutheria; Primates; Catarrhini; Hominidae;
 Homo.
 25 REFERENCE 1 (residues 1 to 269)
 AUTHORS Kawashima,I., Tani,T., Shimoda,K. and Takiguchi,Y.
 TITLE Characterization of pancreatic elastase II cDNAs: two
 elastase II
 mRNAs are expressed in human pancreas
 30 JOURNAL DNA 6 (2), 163-172 (1987)
 MEDLINE 87217962
 REMARK SEQUENCE FROM N.A.
 REFERENCE 2 (residues 1 to 269)
 35 AUTHORS Fletcher,T.S., Shen,W.F. and Largman,C.
 TITLE Primary structure of human pancreatic elastase 2
 determined by
 sequence analysis of the cloned mRNA
 JOURNAL Biochemistry 26 (23), 7256-7261 (1987)
 40 MEDLINE 88107669
 REMARK SEQUENCE FROM N.A.
 [FUNCTION] ACTS UPON ELASTIN.
 [CATALYTIC ACTIVITY] PREFERENTIAL CLEAVAGE: LEU-|-XAA,
 MET-|-XAA
 45 AND PHE-|-XAA. HYDROLYSES ELASTIN.
 [SUBCELLULAR LOCATION] SECRETED.
 [TISSUE SPECIFICITY] PANCREAS.
 [SIMILARITY] BELONGS TO PEPTIDASE FAMILY S1; ALSO
 KNOWN AS THE
 TRYPSIN FAMILY. ELASTASE SUBFAMILY.
 50 FEATURES Location/Qualifiers
 source 1..269
 /organism="Homo sapiens"
 /db_xref="taxon:9606"
 Region 1..16
 55 /region_name="Signal"
 Protein 1..269
 /product="ELASTASE 2A PRECURSOR"
 /EC_number="3.4.21.71"

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      Region      17..28
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5      Region      29..269
                  /region_name="Mature chain"
                  /note="ELASTASE 2A."
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10     Site        73
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15	Bond	bond(155,222)	/bond_type="disulfide"	/note="BY SIMILARITY."
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20	Bond	bond(212,243)	/bond_type="disulfide"	/note="BY SIMILARITY."
25	Site	216	/site_type="active"	/note="CHARGE RELAY SYSTEM (BY SIMILARITY)."

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      61 slianswvlt aahcissrt yrvglgrhnl yvaesgslav svskivvhkd
      wnsnqiskgn
      121 diallklnp vsldtkiqla clppagtilp nnypcyvtgw grlqtngavp
      dvlqqgrllv
35  181 vdyatcssa wwgssvktsm icaggdgvls scngdsggpl ncqasdgrwq
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      241 lgcnyyhkps vftrvsnyid winsviann
      //

40  NP_056933. pancreatic elasta...[gi:7705648]
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      NOV-2000
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      ACCESSION   NP_056933
45  PID          g7705648
      VERSION     NP_056933.1 GI:7705648
      DBSOURCE    REFSEQ: accession NM_015849.1
      KEYWORDS    .
      SOURCE      human.
50  ORGANISM     Homo sapiens
                  Eukaryota; Metazoa; Chordata; Craniata; Vertebrata;
                  Euteleostomi;
                  Mammalia; Eutheria; Primates; Catarrhini; Hominidae;
                  Homo.
55  REFERENCE    1 (residues 1 to 269)
      AUTHORS    Kawashima,I., Tani,T., Shimoda,K. and Takiguchi,Y.
      TITLE      Characterization of pancreatic elastase II cDNAs: two
                  elastase II

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mRNAs are expressed in human pancreas

JOURNAL DNA 6 (2), 163-172 (1987)

MEDLINE 87217962

COMMENT PROVISIONAL REFSEQ: This record has not yet been
 5 subject to final
 NCBI review. The reference sequence was derived from
 M16653.1.

FEATURES

	Location/Qualifiers
10	source 1..269 /organism="Homo sapiens" /db_xref="taxon:9606" /chromosome="12" /map="12"
15	Protein 1..269 /product="pancreatic elastase IIB"
	sig_peptide 1..16 /note="pancreatic elastase IIB signal peptide"
20	Region 28..262 /region_name="Trypsin-like serine protease" /db_xref="CDD:Tryp_SpC" /note="Tryp_SpC"
	mat_peptide 29..269 /product="pancreatic elastase IIB mature peptide"
25	Region 31..262 /region_name="Trypsin" /db_xref="CDD:pfam00089" /note="trypsin"
30	CDS 1..269 /gene="LOC51032" /db_xref="LocusID:51032" /coded_by="NM_015849.1:26..835"

ORIGIN

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 61 slianswvlt aahciyssri yrvmlgqhn1 yvaesgslav svskivvhkd
 wnsnqvskgn
 121 diallklanp vsltldkiqla clppagtilp nnypcyvtgw grlqtngalp
 40 ddlkqgrllv
 181 vdyatcsssg wwgstvktnm icaggdgvic tcngdsggpl ncqasdgrwe
 vhgigsltsv
 241 lgcnyyykps iftrvsnynd winsviann
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45

PRAME

LOCUS NM_006115 2148 bp mRNA PRI 19-JUN-
 50 2001
 DEFINITION Homo sapiens preferentially expressed antigen in
 melanoma (PRAME),
 mRNA.

ACCESSION NM_006115

55 VERSION NM_006115.1 GI:5174640

KEYWORDS .

SOURCE human.

ORGANISM Homo sapiens

Eukaryota; Metazoa; Chordata; Craniata; Vertebrata;
 Euteleostomi;
 Mammalia; Eutheria; Primates; Catarrhini; Hominidae;
 Homo.

5 REFERENCE 1 (bases 1 to 2148)
 AUTHORS Ikeda,H., Lethe,B., Lehmann,F., Van Baren,N.,
 Baurain,J.-F., De
 Smet,C., Chambost,H., Vitale,M., Moretta,A., Boon,T. and
 Coulie,P.G.

10 TITLE Characterization of an antigen that is recognized on a
 melanoma
 showing partial HLA loss by CTL expressing an NK inhibitor
 receptor
 JOURNAL Immunity 6 (2), 199-208 (1997)
 MEDLINE 97199265

15 REFERENCE 2 (bases 1 to 2148)
 AUTHORS Williams JM, Chen GC, Zhu L and Rest RF.
 TITLE Using the yeast two-hybrid system to identify human
 epithelial cell
 20 proteins that bind gonococcal Opa proteins: intracellular
 gonococci
 bind pyruvate kinase via their Opa proteins and
 require host
 pyruvate for growth
 25 JOURNAL Mol. Microbiol. 27 (1), 171-186 (1998)
 MEDLINE 98125741
 PUBMED 9466265

30 REFERENCE 3 (bases 1 to 2148)
 AUTHORS van Baren,N., Chambost,H., Ferrant,A., Michaux,L., Ikeda,H
 Millard,I., Olive,D., Boon,T. and Coulie,P.G.
 TITLE PRAME, a gene encoding an antigen recognized on a
 human melanoma by
 cytolytic T cells, is expressed in acute leukaemia cells
 35 JOURNAL Br. J. Haematol. 102 (5), 1376-1379 (1998)
 MEDLINE 98423996
 PUBMED 9753074

40 REFERENCE 4 (bases 1 to 2148)
 AUTHORS Dunham I, Shimizu N, Roe BA, Chissole S, Hunt AR, Collins JI
 Bruskiewich R, Beare DM, Clamp M, Smink LJ, Ainscough
 R, Almeida
 JP, Babbage A, Bagguley C, Bailey J, Barlow K, Bates
 KN, Beasley O,
 Bird CP, Blakey S, Bridgeman AM, Buck D, Burgess J,
 Burrill WD,
 45 O'Brien KP and et al.
 TITLE The DNA sequence of human chromosome 22
 JOURNAL Nature 402 (6761), 489-495 (1999)
 MEDLINE 20057165
 PUBMED 10591208

50 REMARK Erratum:[published erratum appears in Nature 2000 Apr
 20;404(6780):904]]
 COMMENT REVIEWED REFSEQ: This record has been curated by NCBI
 staff. The
 55 reference sequence was derived from U65011.1.
 Summary: The protein encoded by this gene has a 509
 amino acid
 antigen, lacking a signal sequence, and recognized on
 a human

melanoma cell line by a T-lymphocyte clone. A significant level of this mRNA is detected in normal testis as well as in many melanomas, non-small cell lung carcinomas, sarcomas, head and neck tumors and renal carcinomas. The encoded protein is expressed predominantly in acute leukemias carrying chromosomal abnormalities such as translocation t(8:21), which fuses the AML1 and ETO genes. Its expression shares several characteristics with the expression patterns of MAGE, BAGE, and GAGE gene families, all of which are expressed in tumors. This protein is expressed in a higher proportion of samples than genes of the MAGE, BAGE, and GAGE families, and of these four groups, only this protein is expressed by acute myeloid leukemias.

COMPLETENESS: complete on the 3' end.

25 FEATURES Location/Qualifiers
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 /db_xref="taxon:9606"
 /chromosome="22"
 /map="22q11.22"
 /haplotype="A24 A28 B13 B44 Cw6 Cw7"
 gene 1..2148
 /gene="PRAME"
 /note="MAPE; OIP4"
 /db_xref="LocusID:23532"
 /db_xref="MIM:606021"
 CDS 236..1765
 /gene="PRAME"
 /note="melanoma antigen preferentially
 40 expressed in tumors; Opa-interacting protein OIP4"
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 melanoma"
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 /db_xref="GI:5174641"
 50 /translation="MERRRLWGSIQSRYISMSVWTSPRRRLVELAGQSLLKDEALAIAALELLPREL
 FPPLFMAAFDGRHSQTLKAMVQAWPFTCLPLGVLMKGQHLHLETFKAVLDGLDVLAAQEVPRRWK
 LQVLDLRKNSHQDFWTVWSGNRLASLYSFPEPEAAQPMTKKRKVDGLSTEAEQPFIPVEVLVDLFLK
 EGACDELFSYLIEKVKKKNVLRLLCCKKLKIFAMPMDIKMILKMVQLDSIEDLEVTCTWKLPTLA
 55 KFSPYLGQMINLRRLLSHIHASSYISPEKEEQYIAQFTSQFLSLQCLQALYVDSLFLRGRRLDQL
 LRHVMNPLETSLITNCRLESGDVMHLSQSPSVLSVLSLGVMLTDVSPPEPLQALLERASATLQD
 LVFDECGITDDQLLALLPSLSHCSQLTTLSTFYGNSISISALQSLQLHLIGLSNLTHVLYPVPLESY
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 variation 254

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/allele="T"
/db_xref="dbSNP:13604"
20     variation      1952
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      polyA_site      2130
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      61 cgggacaccc caccgccttc ccaggcgtga cctgtcaaca gcaacttcgc
      ggtgtggtga
40     121 actctctgag gaaaaacccat ttgtattatt actctcagac gtgcgtggca
      acaagtgact
      181 gagacctaga aatccaagcg ttggagggtcc tgaggccagc ctaagtgcgt
      tcaaaaatgga
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45     tgtggacaag
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      361 tgccgccttg gagttgctgc ccaggagct cttcccgcca ctcttcatgg
      cagcctttga
50     421 cgggagacac agccagaccc tgaaggcaat ggtgcaggcc tggcccttca
      cctgcctccc
      481 tctgggagtg ctgatgaagg gacaacatct tcacctggag accttcaaag
      ctgtgcttga
      541 tggacttgat gtgctccttg ccaggagggt tcgccccagg aggtggaaac
55     ttcaagtgct
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      acagggccag

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661 tctgtactca tttccagagc cagaagcagc tcagcccatg acaaagaagc
 gaaaagtaga
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 tagacctggt
 5 781 cctcaaggaa ggtgcctgtg atgaattggt ctccctacctc attgagaaag
 tgaagcgaaa
 841 gaaaaatgta ctacgcctgt gctgtaagaa gctgaagatt tttgcaatgc
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 10 aagtgaacttg
 961 tacctggaag ctaccacact tggcgaaatt ttctccttac ctgggccaga
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 agaaggaaga
 15 1081 gcagtatatc gccaggttca cctctcagtt cctcagtcctg cagtgcctgc
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 1201 ccccttgga accctctcaa taactaactg ccggctttcg gaaggggatg
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 30 tgctgtatcc
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 agcccatcct
 1741 gtgcccctgt ttcatgccta actagctggg tgcacatata aaatgcttca
 ttctgcatac
 1801 ttggacacta aagccaggat gtgcatgcat cttgaagcaa caaagcagcc
 40 acagtttcag
 1861 acaaagtgtc agtgtgagtg aggaaaacat gttcagtgag gaaaaaacat
 tcagacaaat
 1921 gttcagtgag gaaaaaaagg ggaagttggg gataggcaga tgttgacttg
 aggagttaat
 45 1981 gtgatctttg gggagataca tcttatagag ttagaaatag aatctgaatt
 tctaaaggga
 2041 gattctggct tgggaagtac atgtaggagt taatccctgt gtagactgtt
 gtaaagaaac
 2101 tgttgaaaat aaagagaagc aatgtgaagc aaaaaaaaaa aaaaaaaaaa

ED-B domain of Fibronectin

LOCUS HSFIBEDB 2823 bp DNA linear
 PRI 09-AUG-1999

5 DEFINITION Human fibronectin gene ED-B region.
 ACCESSION X07717
 VERSION X07717.1 GI:31406
 KEYWORDS alternate splicing; fibronectin.
 SOURCE human.

10 ORGANISM Homo sapiens
 Eukaryota; Metazoa; Chordata; Craniata; Vertebrata;
 Euteleostomi;
 Mammalia; Eutheria; Primates; Catarrhini; Hominidae;
 Homo.

15 REFERENCE 1 (bases 1 to 2823)
 AUTHORS Paolella,G., Henchcliffe,C., Sebastio,G. and
 Baralle,F.E.
 TITLE Sequence analysis and in vivo expression show that
 alternative
 20 splicing of ED-B and ED-A regions of the human
 fibronectin gene are
 independent events
 JOURNAL Nucleic Acids Res. 16 (8), 3545-3557 (1988)
 MEDLINE 88233940

25 FEATURES Location/Qualifiers
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CEA
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DEFINITION Homo sapiens carcinoembryonic antigen-related cell
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ACCESSION NM_004363
VERSION NM_004363.1 GI:11386170
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Mammalia; Eutheria; Primates; Catarrhini; Hominidae;
15 Homo.
REFERENCE 1 (bases 1 to 2974)
AUTHORS Oikawa S, Nakazato H and Kosaki G.
TITLE Primary structure of human carcinoembryonic antigen
(CEA) deduced
20 from cDNA sequence
JOURNAL Biochem. Biophys. Res. Commun. 142 (2), 511-518 (1987)
MEDLINE 87128144
PUBMED 3814146
REFERENCE 2 (bases 1 to 2974)
25 AUTHORS Zimmermann W, Weber B, Ortlieb B, Rudert F, Schempp W,
Fiebig HH,
Shively JE, von Kleist S and Thompson JA.
TITLE Chromosomal localization of the carcinoembryonic
antigen gene
30 family and differential expression in various tumors
JOURNAL Cancer Res. 48 (9), 2550-2554 (1988)
MEDLINE 88184584
PUBMED 3356015
REFERENCE 3 (bases 1 to 2974)
35 AUTHORS Barnett,T., Goebel,S.J., Nothdurft,M.A. and
Elting,J.J.
TITLE Carcinoembryonic antigen family: characterization of
cDNAs coding

for NCA and CEA and suggestion of nonrandom sequence
variation in
their conserved loop-domains

JOURNAL Genomics 3 (1), 59-66 (1988)

5 MEDLINE 89122014

REFERENCE 4 (bases 1 to 2974)

AUTHORS Barnett T and Zimmermann W.

TITLE Workshop report: proposed nomenclature for the
carcinoembryonic
antigen (CEA) gene family

10 JOURNAL Tumour Biol. 11 (1-2), 59-63 (1990)

MEDLINE 90176333

PUBMED 2309067

REFERENCE 5 (bases 1 to 2974)

15 AUTHORS Schrewe H, Thompson J, Bona M, Hefta LJ, Maruya A,
Hassauer M,
Shively JE, von Kleist S and Zimmermann W.

TITLE Cloning of the complete gene for carcinoembryonic
antigen: analysis
of its promoter indicates a region conveying cell
20 type-specific
expression

JOURNAL Mol. Cell. Biol. 10 (6), 2738-2748 (1990)

MEDLINE 90258861

25 PUBMED 2342461

COMMENT PROVISIONAL REFSEQ: This record has not yet been
subject to final
NCBI review. The reference sequence was derived from
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25

Her2/Neu

LOCUS HUMHER2A 4530 bp mRNA linear
 PRI 18-SEP-1995

30 DEFINITION Human tyrosine kinase-type receptor (HER2) mRNA,
 complete cds.

ACCESSION M11730

VERSION M11730.1 GI:183986

KEYWORDS tyrosine kinase.

35 SOURCE Homo sapiens (clone: lambda-HER2-436) fetal cDNA to
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ORGANISM Homo sapiens

Eukaryota; Metazoa; Chordata; Craniata; Vertebrata;
 Euteleostomi;

Mammalia; Eutheria; Primates; Catarrhini; Hominidae;
Homo.
REFERENCE 1 (bases 1 to 4530)
AUTHORS Coussens, L., Yang-Feng, T.L., Liao, Y.-C., Chen, E.,
5 Gray, A.,
McGrath, J., Seeburg, P.H., Libermann, T.A.,
Schlessinger, J.,
Francke, U., Levinson, A. and Ullrich, A.
TITLE Tyrosine kinase receptor with extensive homology to
10 EGF receptor
shares chromosomal location with neu oncogene
JOURNAL Science 230 (4730), 1132-1139 (1985)
MEDLINE 86070181
REFERENCE 2 (bases 1701 to 1719)
15 AUTHORS Ullrich, A.
JOURNAL Unpublished (1988)
FEATURES Location/Qualifiers
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      REFERENCE  1 (bases 1 to 3393)
      AUTHORS    Meuwissen,R.L., Meerts,I., Hoovers,J.M., Leschot,N.J.
      and
35 Heyting,C.
      TITLE      Human synaptonemal complex protein 1 (SCP1): isolation
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gene

JOURNAL Genomics 39 (3), 377-384 (1997)

MEDLINE 97224467

REFERENCE 2 (bases 1 to 3393)

5 AUTHORS Meuwissen,R.J.L.

TITLE Direct Submission

JOURNAL Submitted (13-FEB-1996) Dr. R.L.J. Meuwissen,

Agricultural

University, Genetics, Dreijenlaan 2, 6703 HA

10 WAGENINGEN,

NETHERLANDS

FEATURES Location/Qualifiers

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10

SSX-4

LOCUS NM_005636 576 bp mRNA linear
 PRI 10-DEC-2001

DEFINITION Homo sapiens synovial sarcoma, X breakpoint 4 (SSX4),
 mRNA.

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ACCESSION NM_005636
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ORGANISM Homo sapiens
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 Euteleostomi;
 Mammalia; Eutheria; Primates; Catarrhini; Hominidae;
 Homo.

25

REFERENCE 1 (bases 1 to 576)
 AUTHORS Gure,A.O., Tureci,O., Sahin,U., Tsang,S.,
 Scanlan,M.J., Knuth,A.,
 Pfreundschuh,M., Old,L.J. and Chen,Y.T.

30

TITLE SSX: a multigene family with several members
 transcribed in normal
 testis and human cancer

JOURNAL Int. J. Cancer 72 (6), 965-971 (1997)

MEDLINE 98021352

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COMMENT PROVISIONAL REFSEQ: This record has not yet been
 subject to final

NCBI review. The reference sequence was derived from
 U90841.1.

FEATURES Location/Qualifiers
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10 All patents and publications mentioned in the specification
are indicative of the levels of those skilled in the art to which
the invention pertains. All patents and publications are herein
incorporated by reference to the same extent as if each individual
publication was specifically and individually indicated to be
incorporated by reference.

15 The invention illustratively described herein suitably may be
practiced in the absence of any element or elements, limitation or
limitations which is not specifically disclosed herein. The terms
and expressions which have been employed are used as terms of
description and not of limitation, and there is no intention that
20 in the use of such terms and expressions indicates the exclusion
of equivalents of the features shown and described or portions
thereof. It is recognized that various modifications are possible
within the scope of the invention claimed. Thus, it should be
understood that although the present invention has been
25 specifically disclosed by preferred embodiments and optional
features, modification and variation of the concepts herein
disclosed may be resorted to by those skilled in the art, and that
such modifications and variations are considered to be within the
scope of this invention as defined by the appended claims.

30

WHAT IS CLAIMED IS:

1. An isolated epitope, comprising a component selected from the group consisting of:
 - (i) a polypeptide having the sequence as disclosed in TABLE 1;
 - (ii) an epitope cluster comprising the polypeptide of (i);
 - (iii) a polypeptide having substantial similarity to (i) or (ii);
 - (iv) a polypeptide having functional similarity to any of (i) through (iii); and
 - (v) a nucleic acid encoding the polypeptide of any of (i) through (iv).
2. The epitope of claim 1, wherein the epitope is immunologically active.
3. The epitope of claim 1, wherein the polypeptide is less than about 30 amino acids in length.
4. The epitope of claim 1, wherein the polypeptide is 8 to 10 amino acids in length.
5. The epitope of claim 1, wherein the substantial or functional similarity comprises addition of at least one amino acid.
6. The epitope of claim 5, wherein the at least one additional amino acid is at an N-terminus of the polypeptide.
7. The epitope of claim 1, wherein the substantial or functional similarity comprises a substitution of at least one amino acid.
8. The epitope of claim 1, the polypeptide having affinity to an HLA-A2 molecule.
9. The epitope of claim 8, wherein the affinity is determined by an assay of binding.
10. The epitope of claim 8, wherein the affinity is determined by an assay of restriction of epitope recognition.
11. The epitope of claim 8, wherein the affinity is determined by a prediction algorithm.
12. The epitope of claim 1, the polypeptide having affinity to an HLA-B7 or HLA-B51 molecule.
13. The epitope of claim 1, wherein the polypeptide is a housekeeping epitope.
14. The epitope of claim 1, wherein the polypeptide corresponds to an epitope displayed on a tumor cell.
15. The epitope of claim 1, wherein the polypeptide corresponds to an epitope displayed on a neovasculature cell.
16. The epitope of claim 1, wherein the peptide is an immune epitope.
17. The epitope of claim 1 wherein the epitope is a nucleic acid.
18. A pharmaceutical composition comprising the peptide of claim 1 and a pharmaceutically acceptable adjuvant, carrier, diluent, or excipient.
19. The composition of claim 18, where the adjuvant is a polynucleotide.

20. The composition of claim 19 wherein the polynucleotide comprises a dinucleotide.
21. The composition of claim 20 wherein the dinucleotide is CpG.
22. The composition of claim 18, wherein the adjuvant is encoded by a polynucleotide.
23. The composition of claim 18 wherein the adjuvant is a cytokine.
- 5 24. The composition of claim 23 wherein the cytokine is GM-CSF.
25. The composition of claim 18 further comprising a professional antigen-presenting cell (pAPC).
26. The composition of claim 25, wherein the pAPC is a dendritic cell.
27. The composition of claim 18, further comprising a second epitope.
- 10 28. The composition of claim 27, wherein the second epitope is a polypeptide.
29. The composition of claim 27, wherein the second epitope is a nucleic acid.
30. The composition of claim 27, wherein the second epitope is a housekeeping epitope.
31. The composition of claim 27, wherein the second epitope is an immune epitope.
- 15 32. A pharmaceutical composition comprising the nucleic acid of claim 1 and a pharmaceutically acceptable adjuvant, carrier, diluent, or excipient.
33. A recombinant construct comprising the nucleic acid of Claim 1.
34. The construct of claim 33, further comprising a plasmid, a viral vector, or an artificial chromosome.
- 20 35. The construct of claim 33, further comprising a sequence encoding at least one feature selected from the group consisting of a second epitope, an IRES, an ISS, an NIS, and ubiquitin.
36. A purified antibody that specifically binds to the epitope of claim 1.
37. A purified antibody that specifically binds to a peptide-MHC protein complex
- 25 comprising the epitope of claim 1.
38. The antibody of claim 36 or claim 37, wherein the antibody is a monoclonal antibody.
39. A multimeric MHC-peptide complex comprising the epitope of claim 1.
40. An isolated T cell expressing a T cell receptor specific for an MHC-peptide
- 30 complex, the complex comprising the epitope of claim 1.
41. The T cell of claim 40, produced by an *in vitro* immunization.
42. The T cell of claim 40, isolated from an immunized animal.
43. A T cell clone comprising the T cell of claim 40.
44. A polyclonal population of T cells comprising the T cell of claim 40.
- 35 45. A pharmaceutical composition comprising the T cell of claim 40 and a pharmaceutically acceptable adjuvant, carrier, diluent, or excipient.

46. An isolated protein molecule comprising the binding domain of a T cell receptor specific for an MHC-peptide complex, the complex comprising the epitope of claim 1.
47. The protein of claim 46, wherein the protein is multivalent.
48. An isolated nucleic acid encoding the protein of claim 46.
- 5 49. A recombinant construct comprising the nucleic acid of claim 48.
50. A host cell expressing the recombinant construct of claim 33 or 49.
51. The host cell of claim 50, wherein the host cell is a dendritic cell, macrophage, tumor cell, or tumor-derived cell.
52. The host cell of claim 50, wherein the host cell is a bacterium, fungus, or
10 protozoan.
53. A pharmaceutical composition comprising the host cell of claim 50 and a pharmaceutically acceptable adjuvant, carrier, diluent, or excipient.
54. A vaccine or immunotherapeutic composition comprising at least one component selected from the group consisting of the epitope of claim 1; the composition of claim 18, 32, 45, or
15 53; the construct of claim 33; the T cell of claim 40, and the host cell of claim 50.
55. A method of treating an animal, comprising:
administering to an animal the vaccine or immunotherapeutic composition of claim
54.
56. The method of claim 55, wherein the administering step comprises a mode of
20 delivery selected from the group consisting of transdermal, intranodal, perinodal, oral, intravenous, intradermal, intramuscular, intraperitoneal, mucosal, aerosol inhalation, and instillation.
57. The method of claim 55, further comprising a step of assaying to determine a characteristic indicative of a state of a target cell or target cells.
58. The method of claim 57, comprising a first assaying step and a second assaying
25 step, wherein the first assaying step precedes the administering step, and wherein the second assaying step follows the administering step.
59. The method of claim 58, further comprising a step of comparing the characteristic determined in the first assaying step with the characteristic determined in the second assaying step to obtain a result.
- 30 60. The method of claim 59, wherein the result is selected from the group consisting of: evidence of an immune response, a diminution in number of target cells, a loss of mass or size of a tumor comprising target cells, a decrease in number or concentration of an intracellular parasite infecting target cells.
61. A method of evaluating immunogenicity of a vaccine or immunotherapeutic
35 composition, comprising:

- administering to an animal the vaccine or immunotherapeutic composition of claim 54; and
- evaluating immunogenicity based on a characteristic of the animal.
62. The method of claim 61, wherein the animal is HLA-transgenic.
- 5 63. A method of evaluating immunogenicity, comprising:
in vitro stimulation of a T cell with the vaccine or immunotherapeutic composition of claim 54; and
evaluating immunogenicity based on a characteristic of the T cell.
64. The method of claim 63, wherein the stimulation is a primary stimulation.
- 10 65. A method of making a passive/adoptive immunotherapeutic, comprising:
combining the T cell of claim 40 or the host cell of claim 50 with a pharmaceutically acceptable adjuvant, carrier, diluent, or excipient.
66. A method of determining specific T cell frequency comprising the step of contacting T cells with a MHC-peptide complex comprising the epitope of claim 1.
- 15 67. The method of claim 66, wherein the contacting step comprises at least one feature selected from the group consisting of immunization, restimulation, detection, and enumeration.
68. The method of Claim 66, further comprising ELISPOT analysis, limiting dilution analysis, flow cytometry, *in situ* hybridization, the polymerase chain reaction or any combination thereof.
- 20 69. A method of evaluating immunologic response, comprising the method of claim 66 carried out prior to and subsequent to an immunization step.
70. A method of evaluating immunologic response, comprising:
determining frequency, cytokine production, or cytolytic activity of T cells, prior to and subsequent to a step of stimulation with MHC-peptide complexes comprising the epitope of claim 1.
- 25 71. A method of diagnosing a disease comprising:
contacting a subject tissue with at least one component selected from the group consisting of the T cell of claim 40, the host cell of claim 50, the antibody of claim 36, the protein of claim 46; and
diagnosing the disease based on a characteristic of the tissue or of the component.
- 30 72. The method of claim 71, wherein the contacting step takes place *in vivo*.
73. The method of claim 71, wherein the contacting step takes place *in vitro*.
74. A method of making a vaccine, comprising:
combining at least one component selected from the group consisting of the epitope of claim 1; the composition of claim 18, 32, 45, or 53; the construct of claim 33;
- 35

the T cell of claim 40, and the host cell of claim 50, with a pharmaceutically acceptable adjuvant, carrier, diluent, or excipient.

5 75. A computer readable medium having recorded thereon the sequence of any one of SEQ ID NOS: X -Y, in a machine having a hardware or software that calculates the physical, biochemical, immunologic, or molecular genetic properties of a molecule embodying said sequence.

 76. A method of treating an animal comprising combining the method of claim 55 combined with at least one mode of treatment selected from the group of radiation therapy, chemotherapy, biochemotherapy, and surgery.

10 77. An isolated polypeptide comprising an epitope cluster from a target-associated antigen having the sequence as disclosed in Tables 25-44, wherein the amino acid sequence consists of not more than about 80% of the amino acid sequence of the antigen.

 78. A vaccine or immunotherapeutic product comprising the polypeptide of claim 78.

 79. An isolated polynucleotide encoding the polypeptide of claim 78.

15 80. A vaccine or immunotherapeutic product comprising the polynucleotide of claim 80.

 81. The polynucleotide of claim 79 or 80, wherein the polynucleotide is DNA.

 82. The polynucleotide of claim 79 or 80, wherein the polynucleotide is RNA.

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FIG. 1B

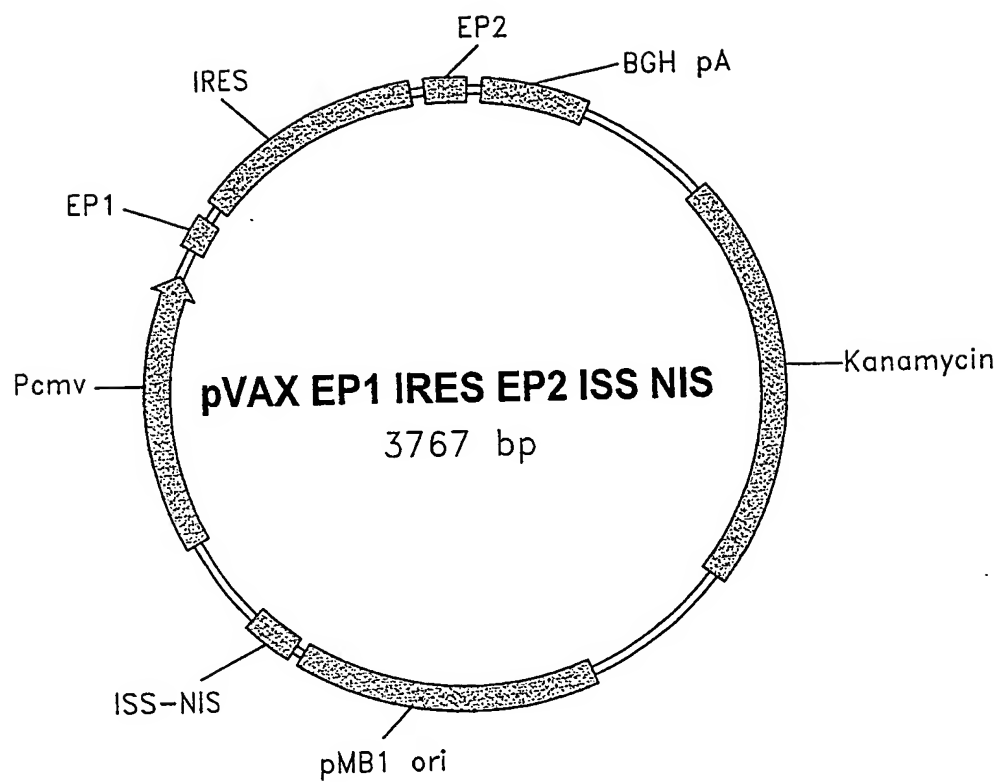
CTAG_HUMAN	NY-ESO	(101)	101	150
AAD05202 - CAG-3		(101)		
CAA11044 -LAGE-1a		(101)		
CAA10194 - LAGE-1s		(101)		
CAA11043 - LAGE-1b		(101)		
CAA10196 - LAGE-1L		(101)		
AAH02833 CT-2		(101)		
Consensus		(101)		
CTAG_HUMAN	NY-ESO	(151)	151	200
AAD05202 - CAG-3		(151)		
CAA11044 -LAGE-1a		(151)		
CAA10194 - LAGE-1s		(151)		
CAA11043 - LAGE-1b		(151)		
CAA10196 - LAGE-1L		(151)		
AAH02833 CT-2		(151)		
Consensus		(151)		

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FIG. 1C

CTAG_HUMAN	NY-ESO	(181)	-----
AA05202	- CAG-3	(181)	-----
CAA11044	-LAGE-1a	(181)	-----
CAA10194	- LAGE-1s	(181)	-----
CAA11043	- LAGE-1b	(201)	FNVMFSAPHI
CAA10196	- LAGE-1L	(201)	FNVMFSAPHI
AAH02833	CT-2	(201)	FNVMFSAPHI
	Consensus	(201)	

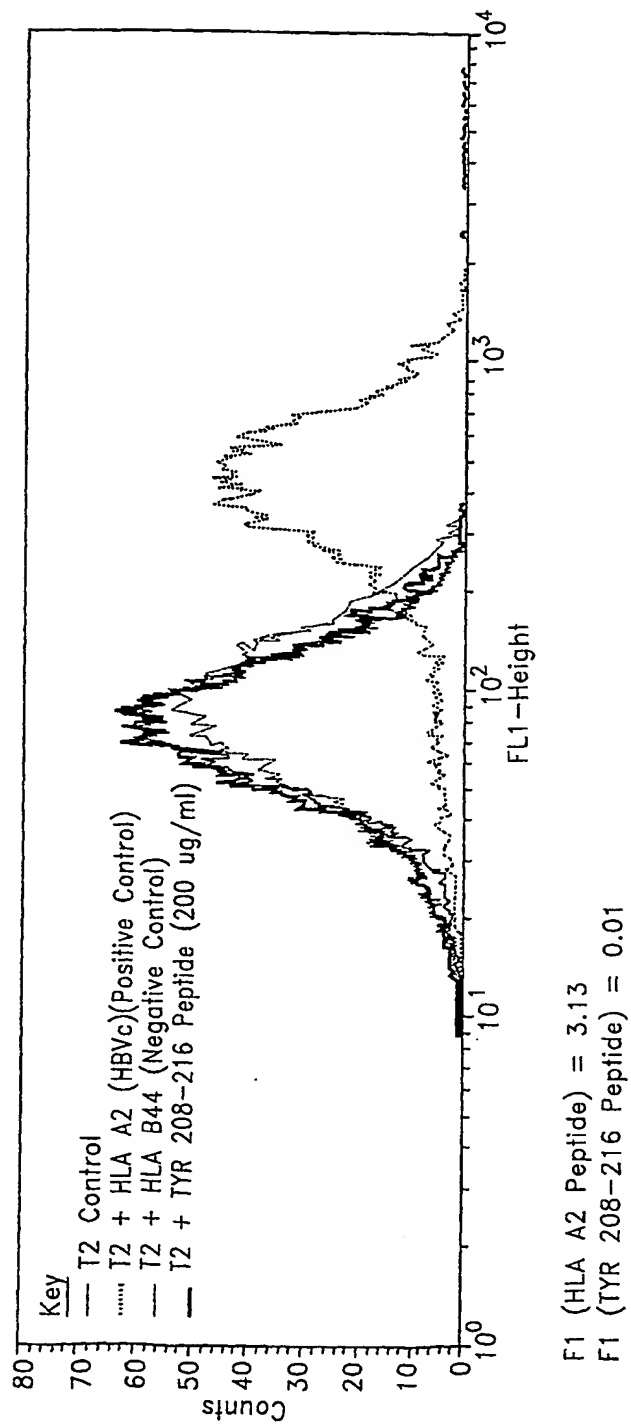
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*FIG. 2*

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FIG. 3A

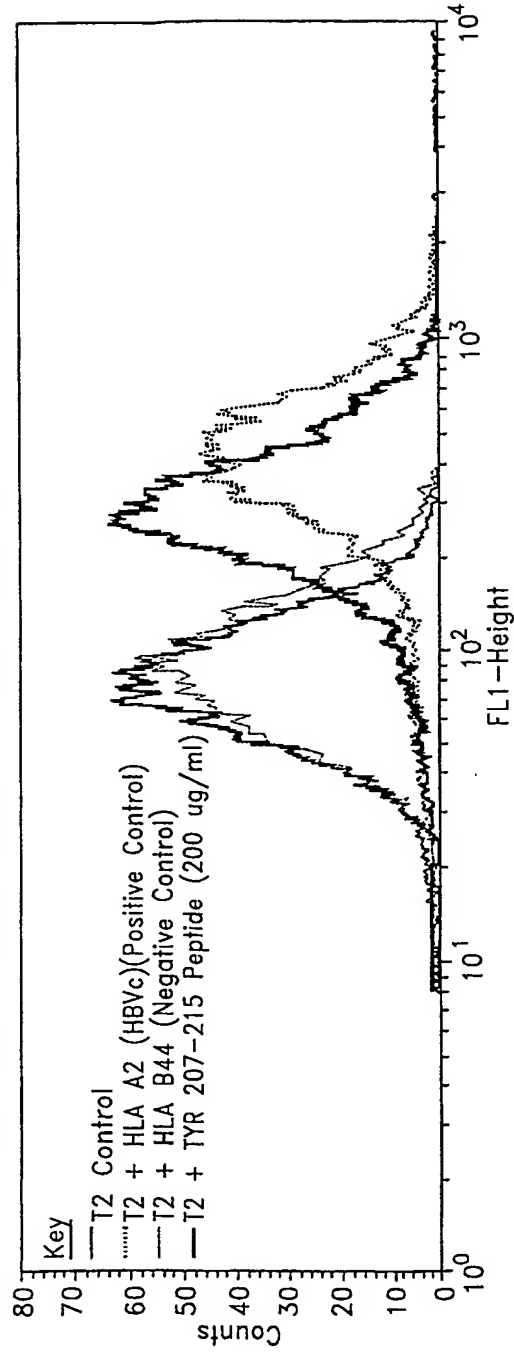
**FACscan Analysis of Binding Assay to Determine the Binding
Ability of Tyrosinase 208-216 Peptide to MHC Class 1**



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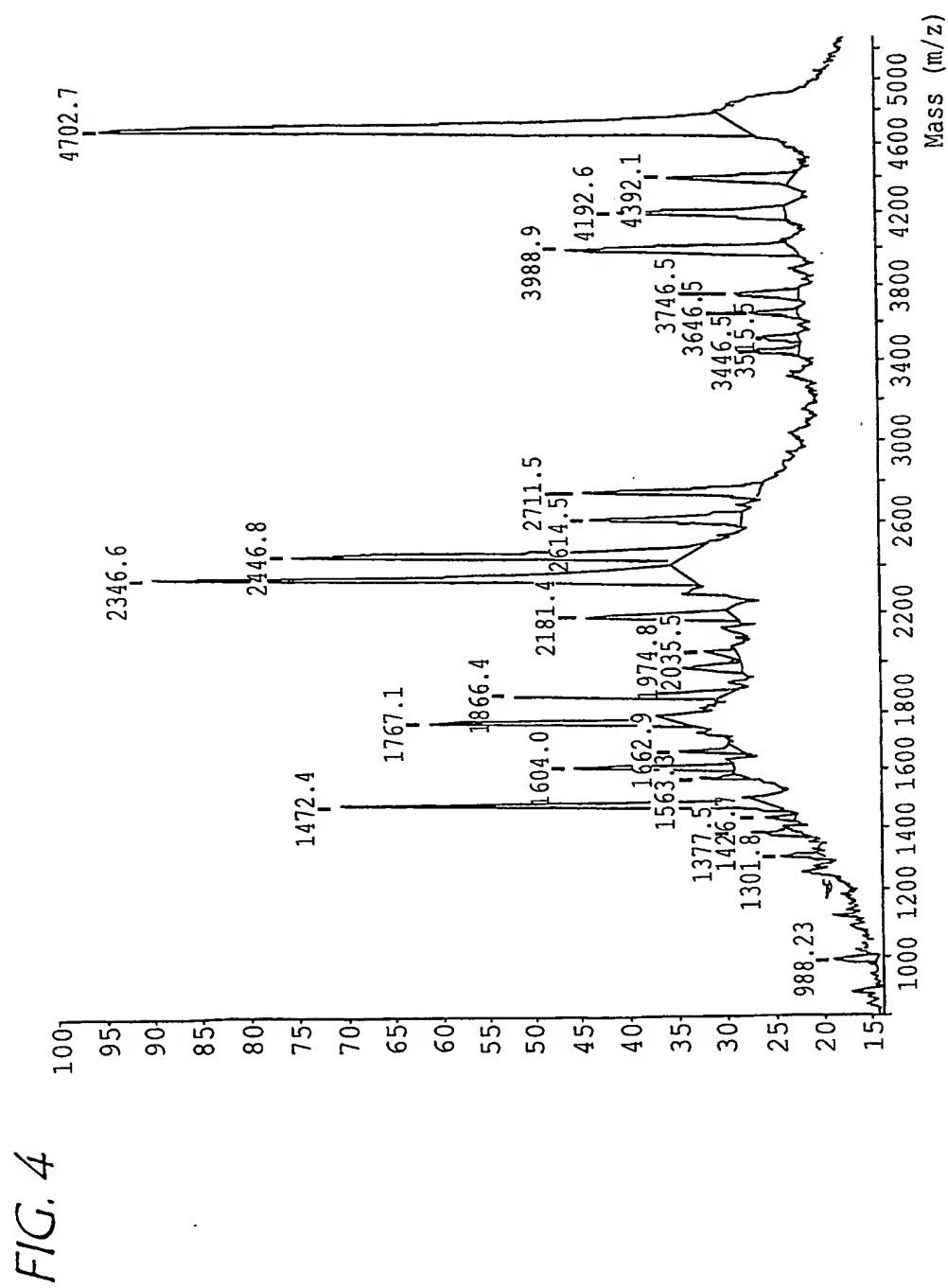
FIG. 3B

FACscan Analysis of Binding Assay to Determine the Binding Ability of Tyrosinase 207-215 Peptide to MHC Class 1



F1 (HLA A2 Peptide) = 3.13
F1 (TYR 207-215 Peptide) = 2.00

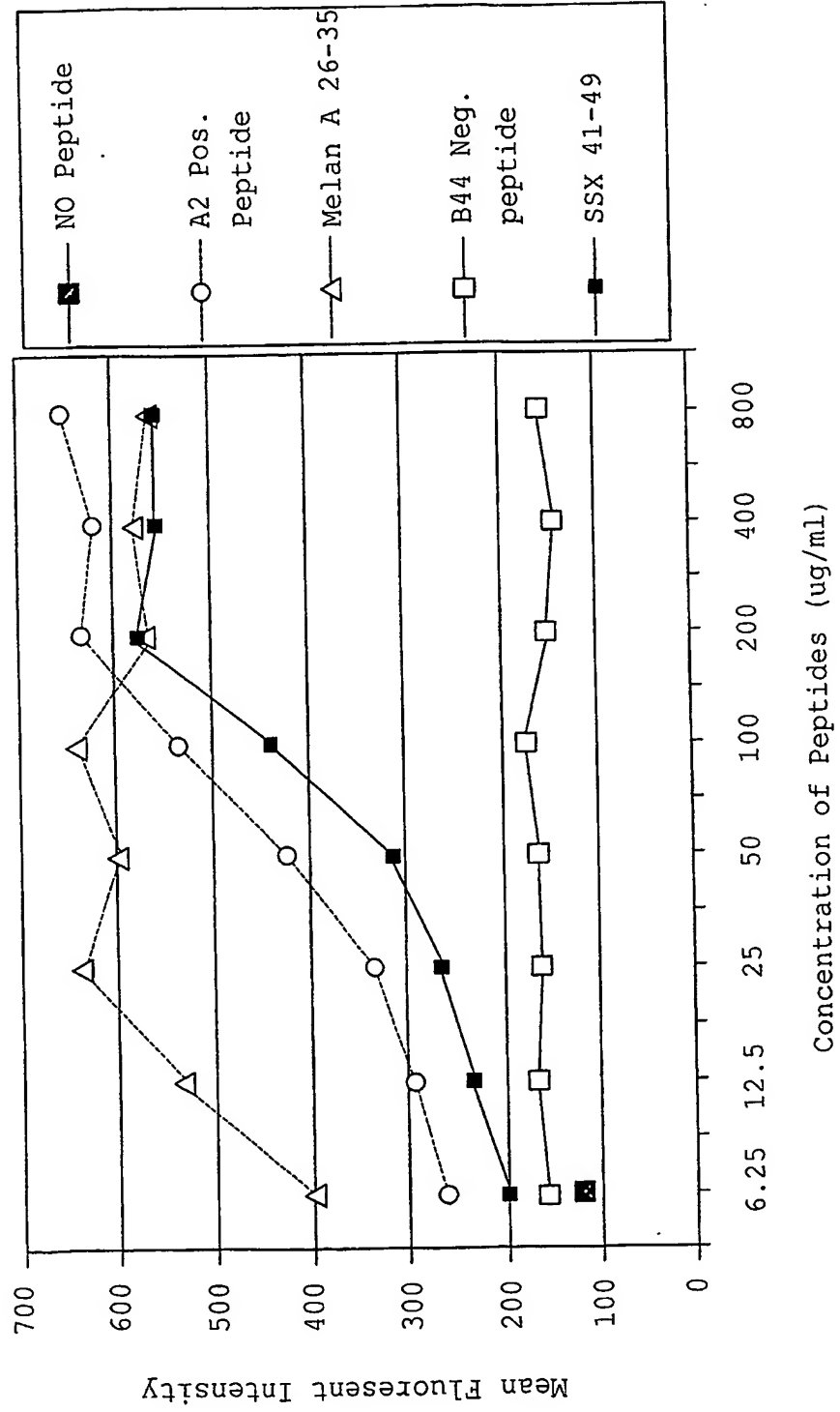
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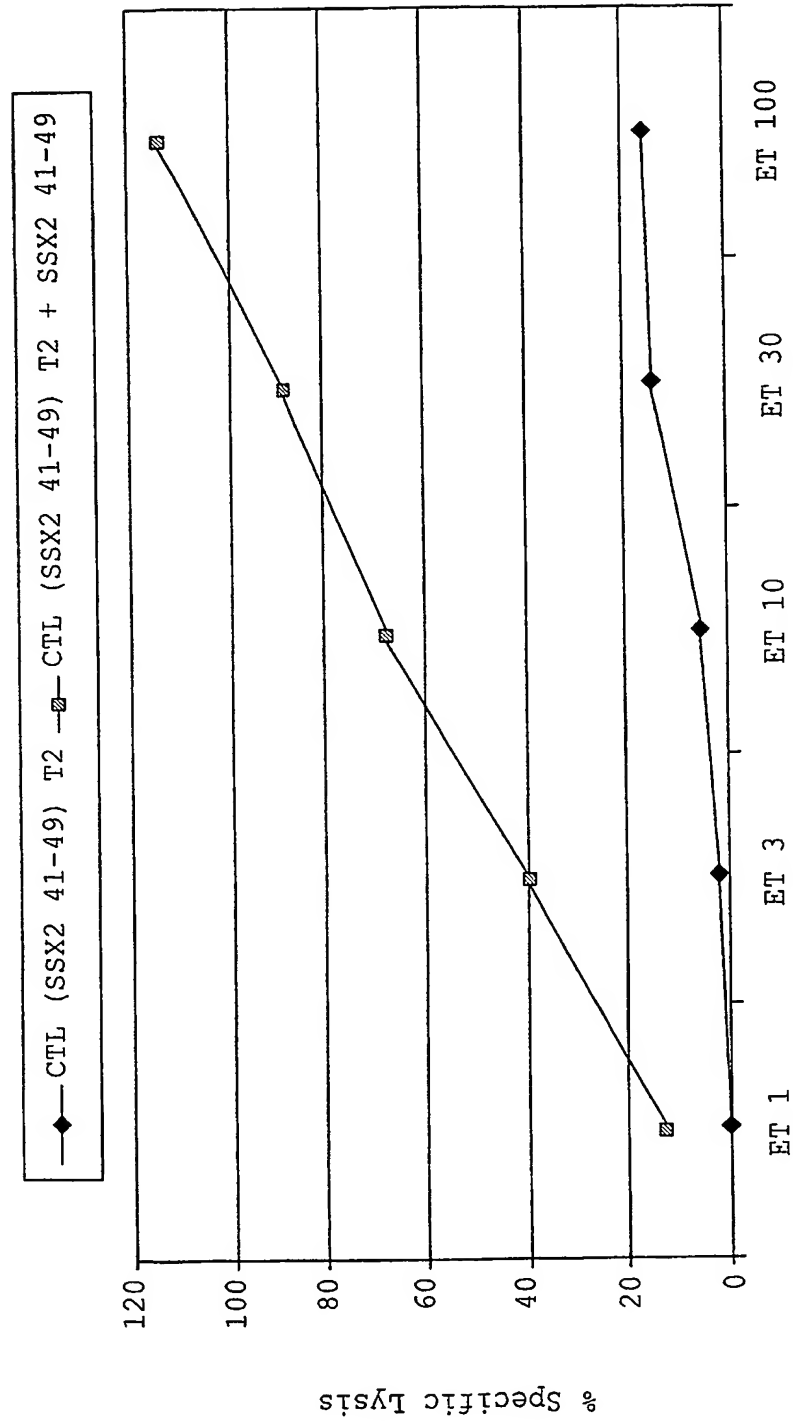
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FIG. 5
Comparison of Peptides Binding Affinity to HLA A2



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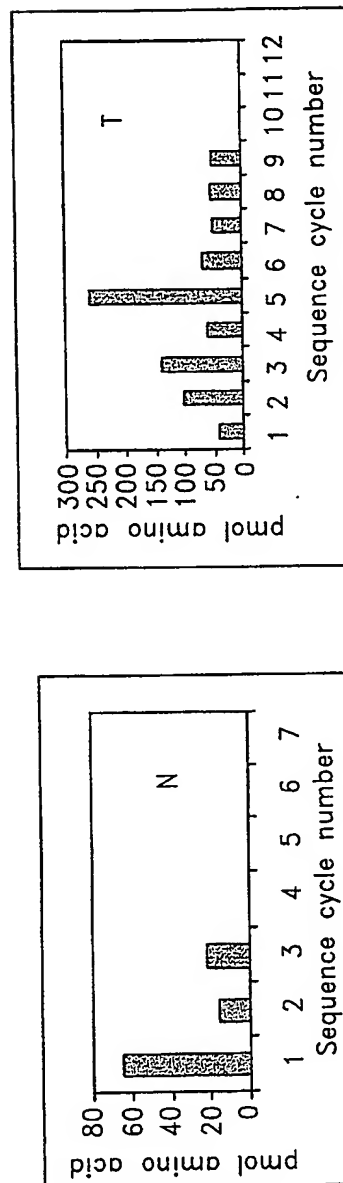
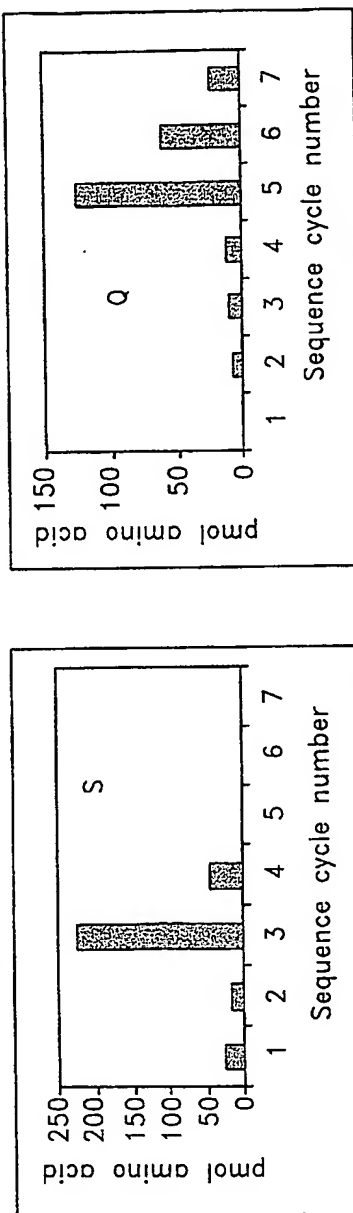
FIG. 6
SSX2₄₁₋₄₉ specific lysis by CTL from peptide
injected HHD1 mice



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FIG. 7A

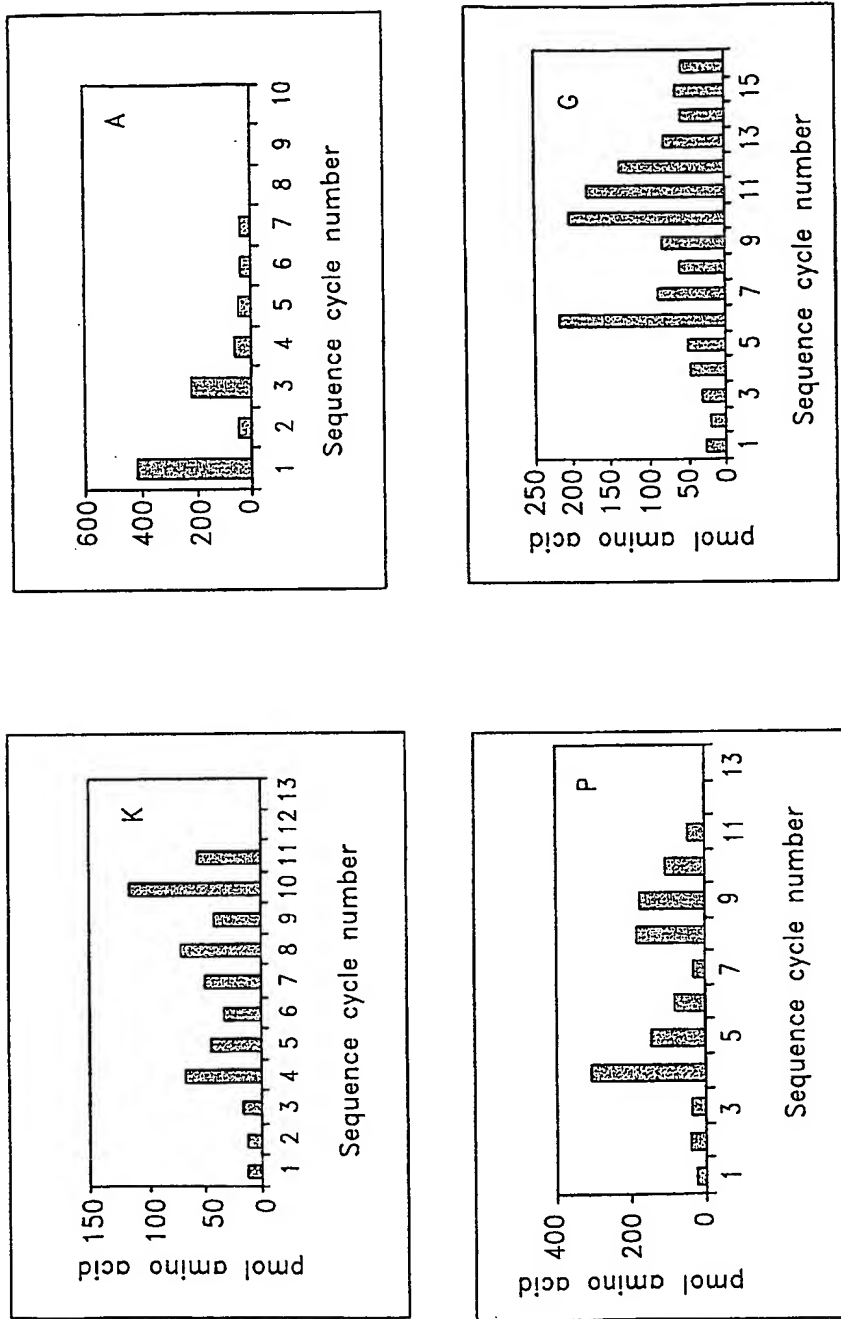
163-AF**SP**QGMPEGLVYV**N**YARTEDEFFKLERDM-192



Pool sequencing of PSMA_163-192 Digested for 60 min by proteasome

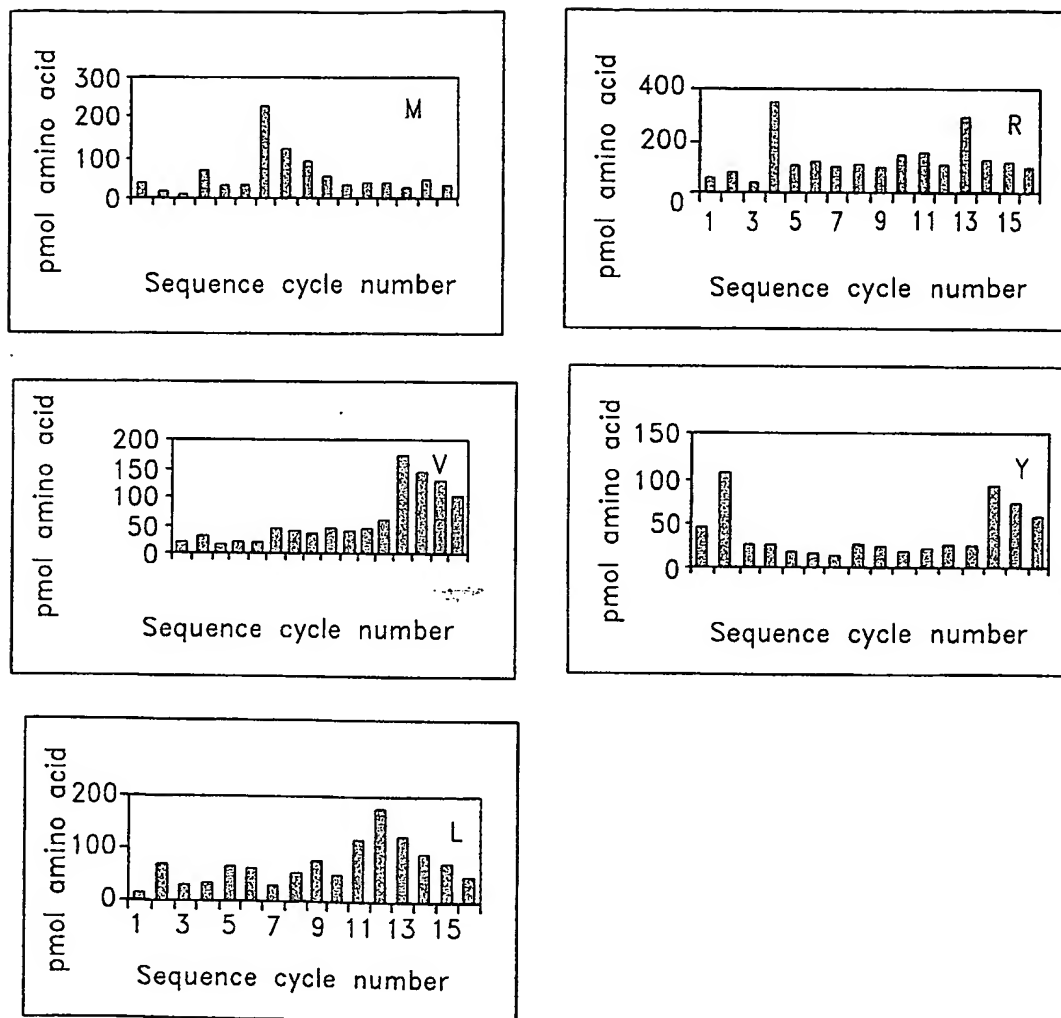
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FIG. 7B



Pool sequencing of PSMA_163-192 Digested for 60 min by proteasome

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Pool sequencing of PSMA_163-192 Digested for 60 min by proteasome

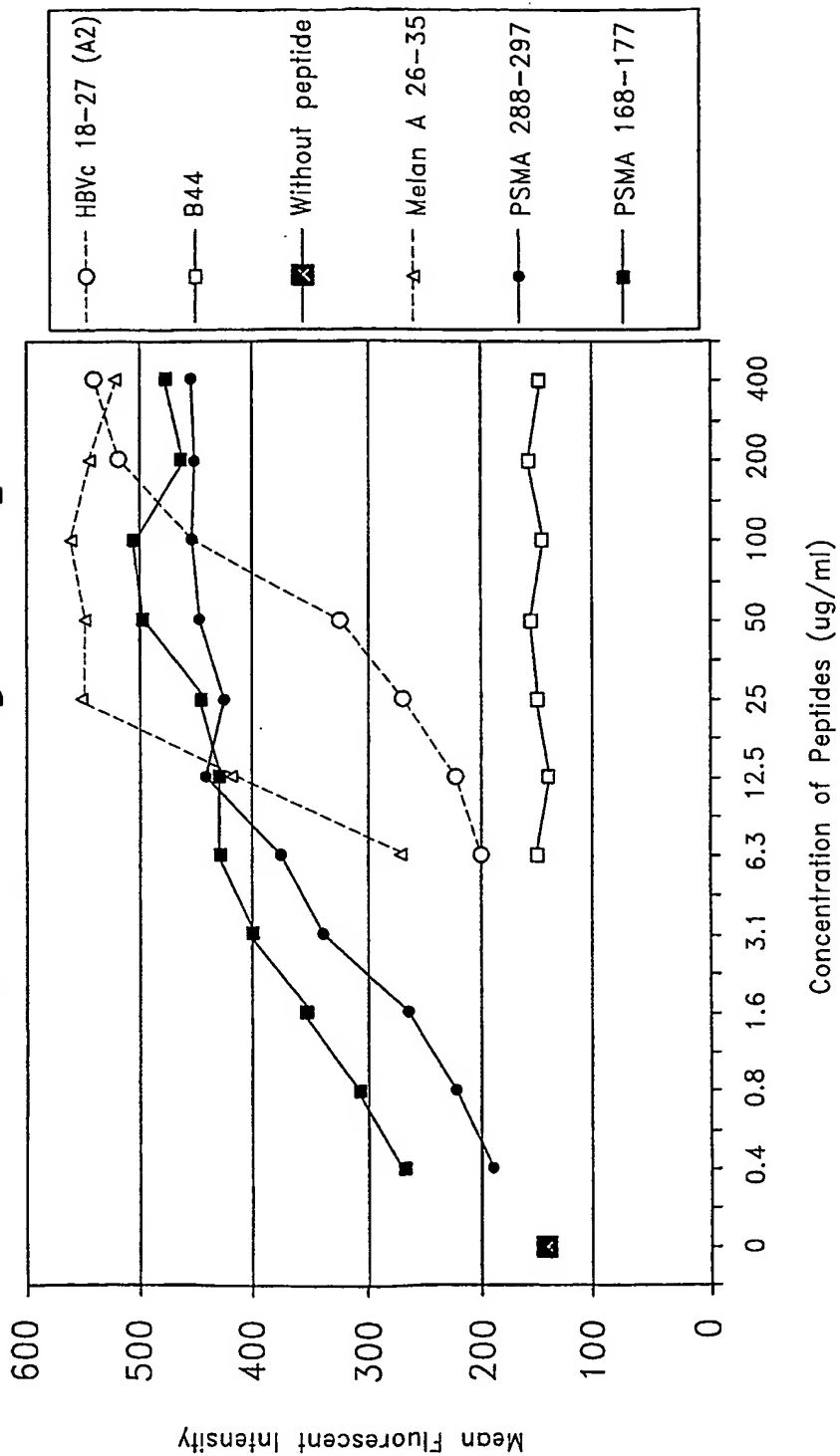
FIG. 7C

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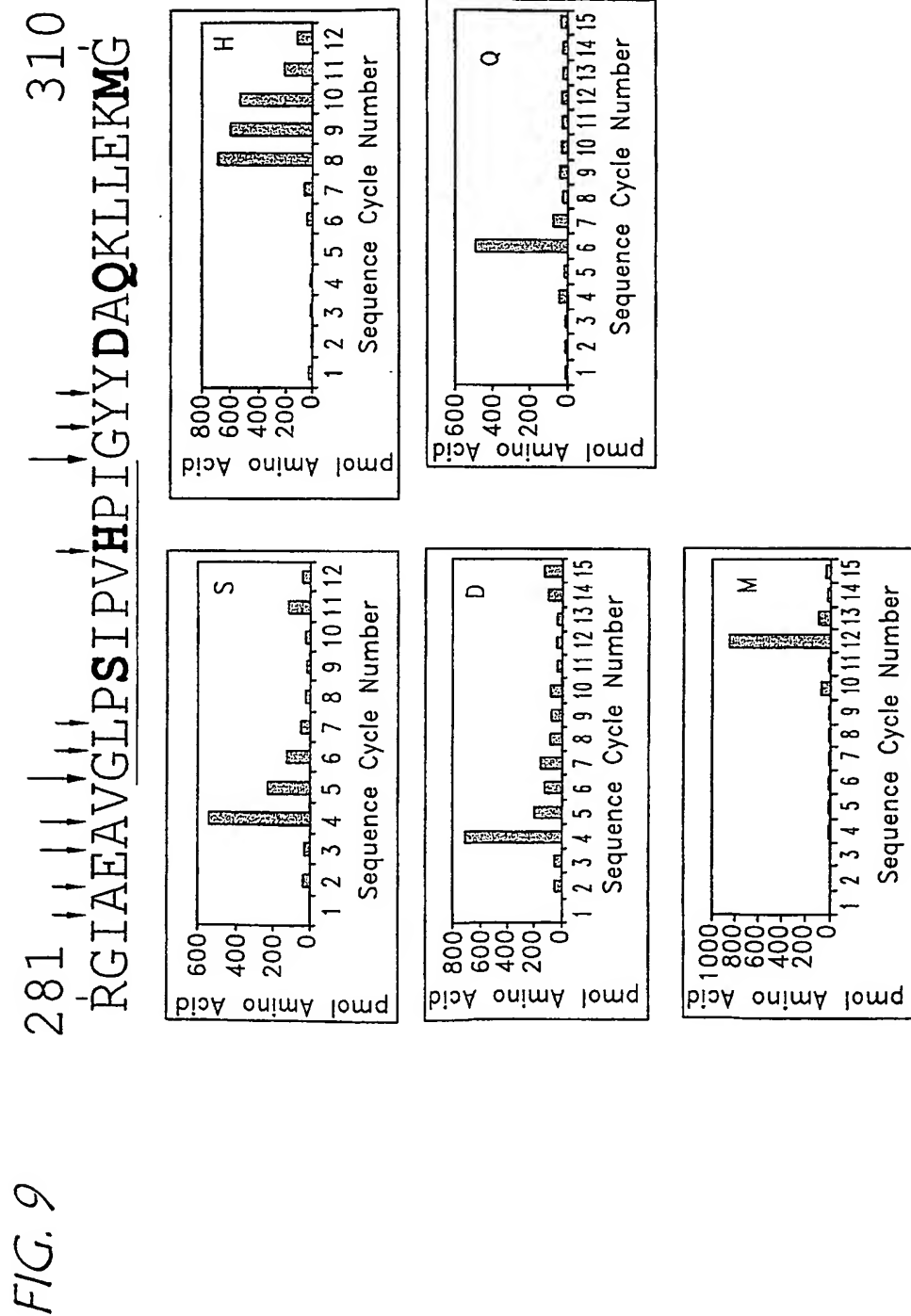
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FIG. 8

HLA A2 Binding Assay



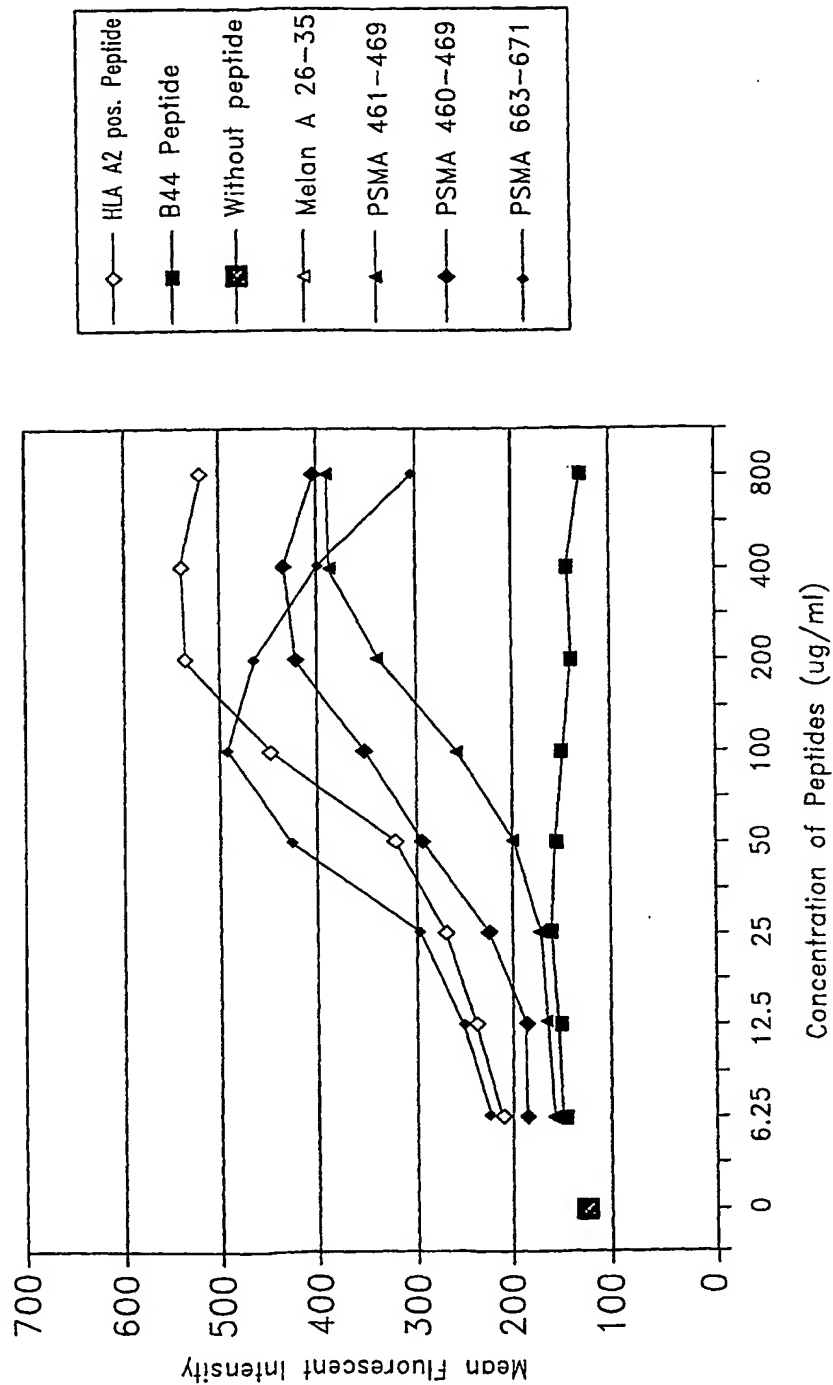
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Pool sequencing of PSMA_281_310 Digested for 60 min by Proteasome

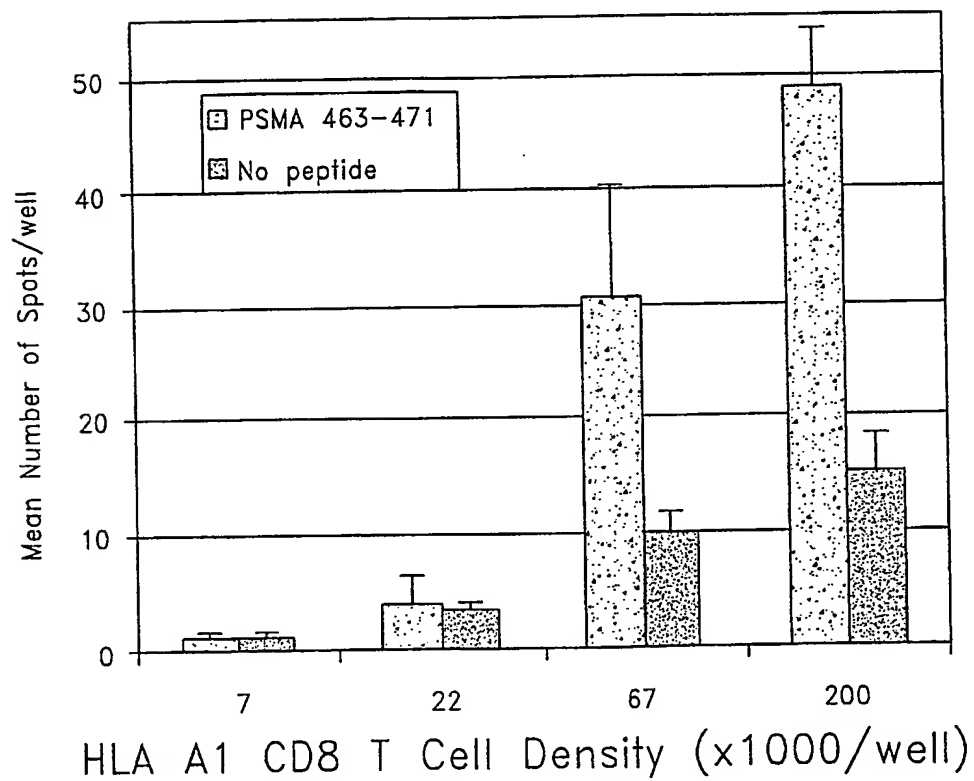
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FIG. 10
Comparison of Peptides Binding
Affinity to HLA A2
by Binding Assay



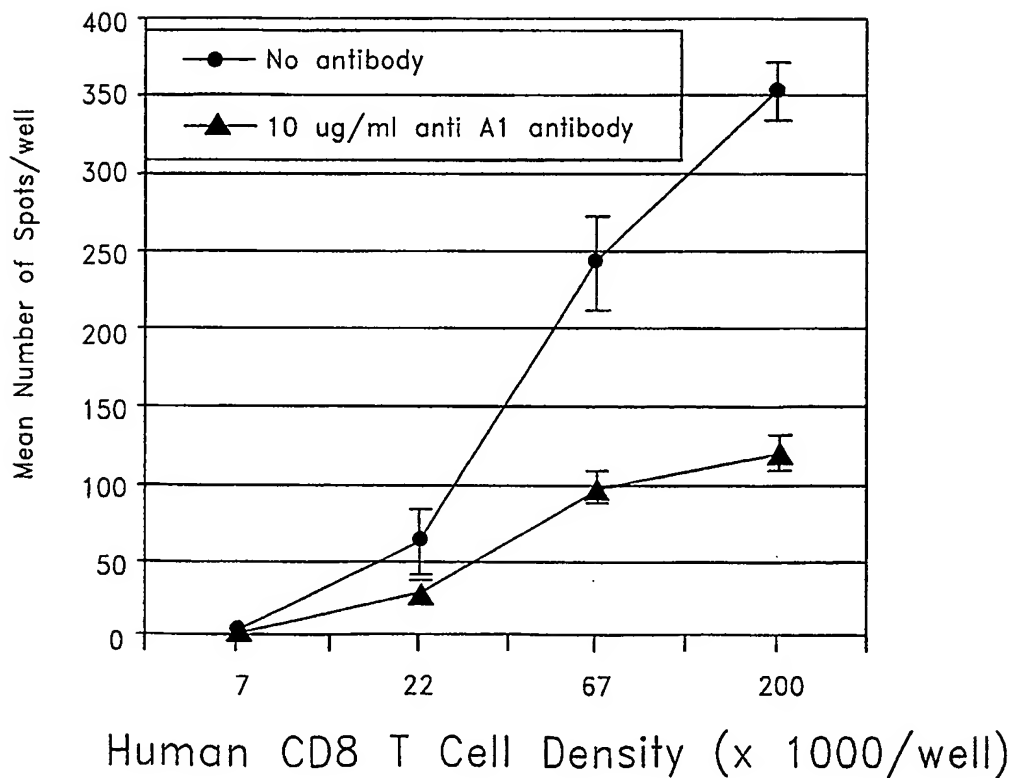
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Autologous DC Present A1 Peptide to CD8 T cell

*FIG. 11*

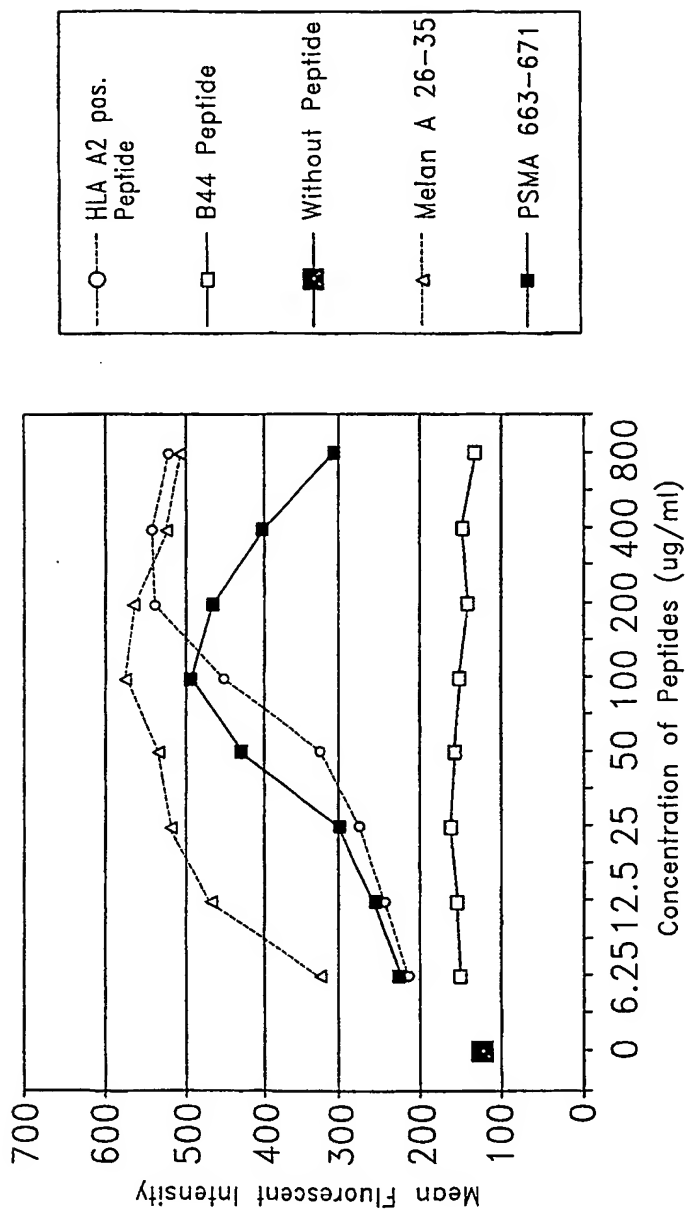
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Secretion of IFN γ Was Blocked by Anti-A1 Antibody

*FIG. 12*

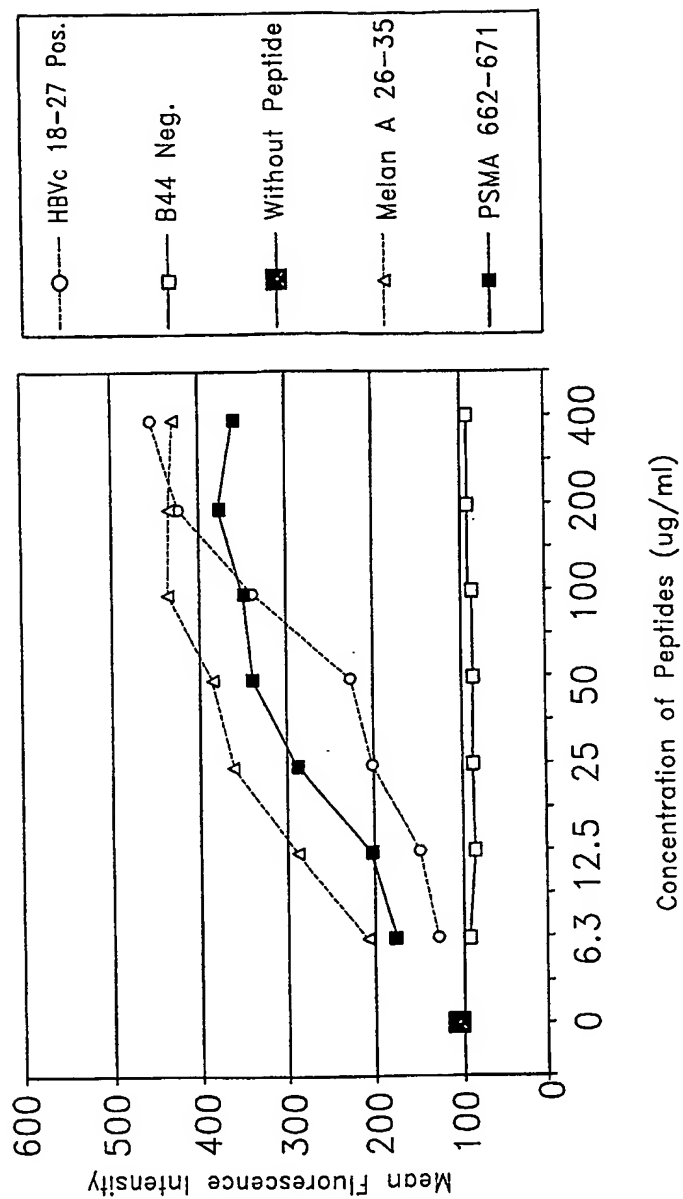
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FIG. 13
Comparison of Peptides Binding Affinity
to HLA A2 by Binding Assay

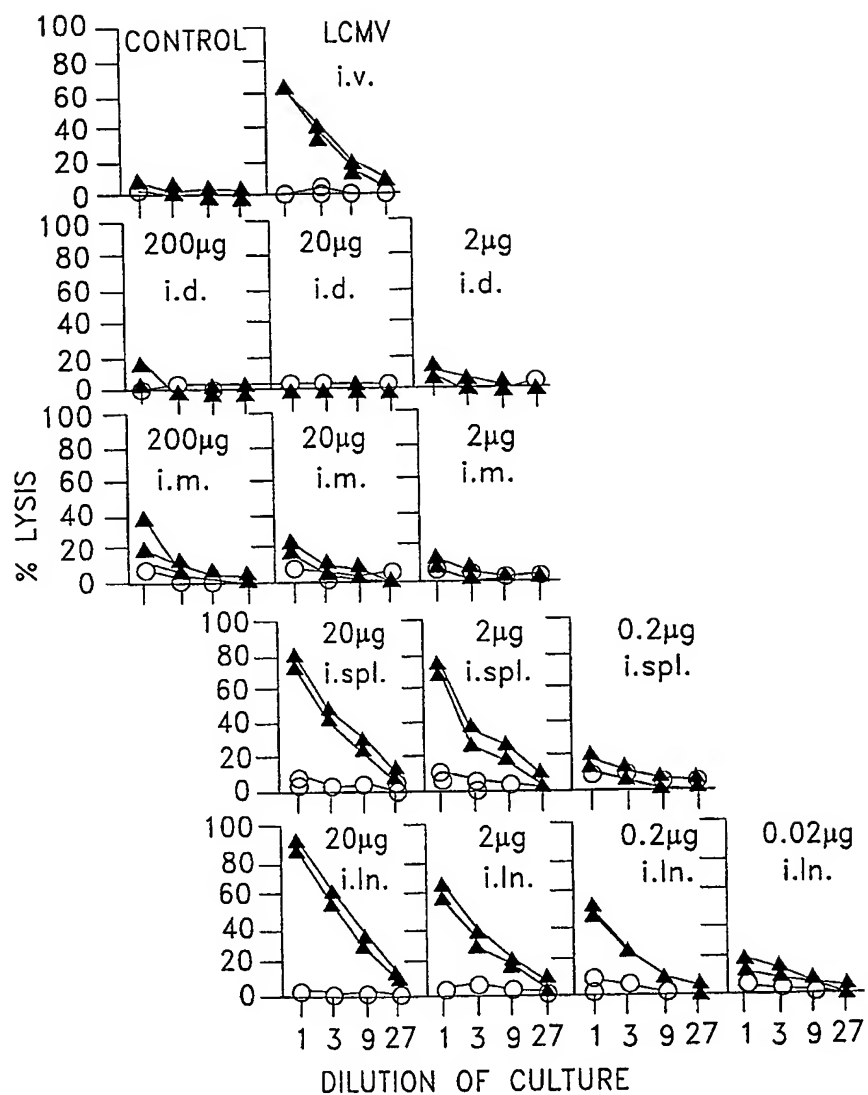


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FIG. 14
Comparison of Peptides Binding Affinity
to HLA A2 by Binding Assay



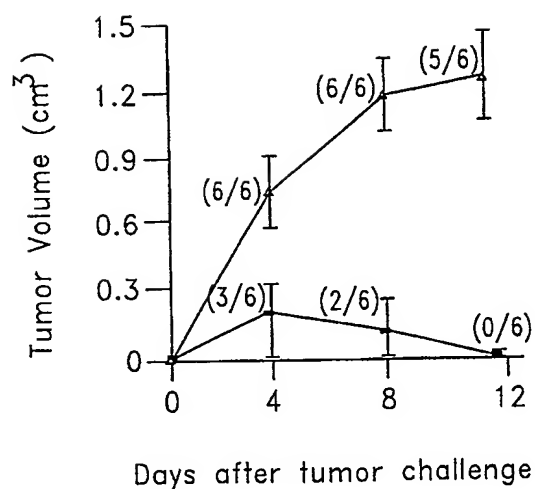
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Graphs show lysis of unpulsed EL4 cells (open circles) and EL4 cells pulsed with gp33 peptide (solid triangles). Symbols represent individual mice and one of three similar experiments is shown.

FIG. 15

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Mean tumor volumes \pm 1SD are shown for mice immunized with pEFGPL33A DNA (solid circles) or control pEGFP-N3 DNA (open triangles). Numbers in brackets indicate number of mice with tumors/total number of mice in group. One of two similar experiments is shown.

FIG. 16

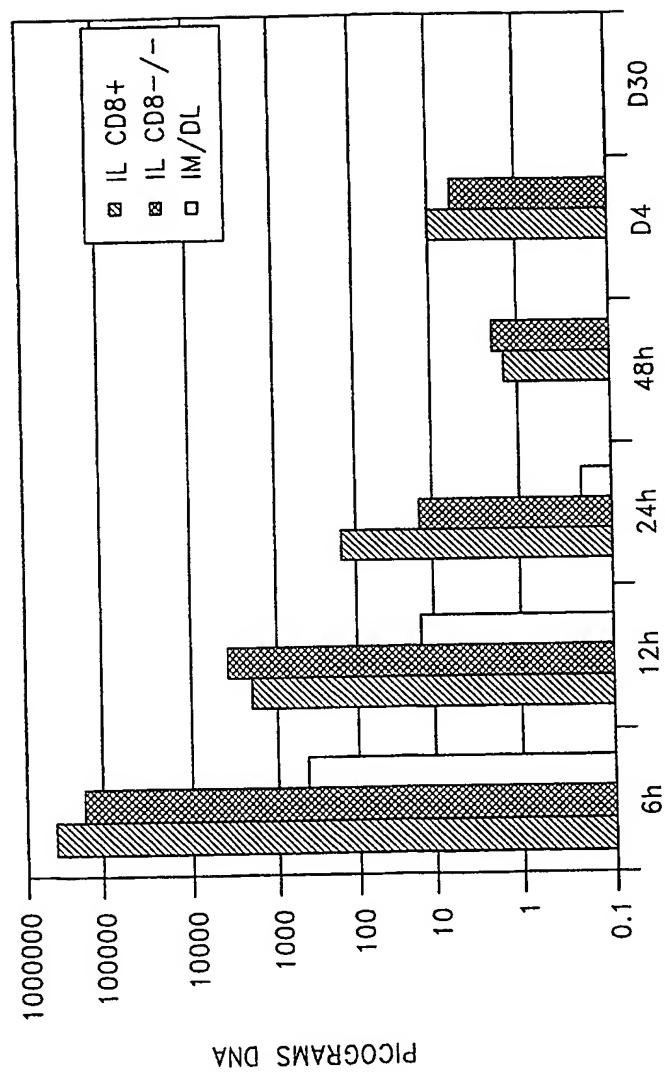


FIG. 17

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